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(54) Title: INHIBITORS OF IASPP INTERACTION WITH AN IASPP BINDING PARTNER AND METHODS OF USE

(57) Abstract: The invention provides the crystal structure of the iASPP C-terminal protein molecule. The structure is set out in Table 1. The structure may be used in to model the interaction of compounds such as pharmaceuticals with this protein, and to determine the structure of related iASPP C-terminal molecules.

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Inhibitors of iASPP interaction with an iASPP Binding Partner and Methods of UseCross-Reference to Related Application

[0001] This application claims the benefit of priority of United Kingdom Patent Application No. 0716322.3, filed August 21, 2007, the disclosure of which is incorporated herein by reference in its entirety.

Field of the Invention

[0002] The present invention relates to the inhibitory apoptosis stimulating protein of p53 (iASPP), crystals of its p53-interacting domain, use of the structure of such crystals and novel peptides that antagonize the function of iASPP.

Background to the Invention

[0003] The tumor suppressor protein p53, the prototypic member of the p53 family, is a transcription factor that responds to oncogenic stress such as DNA damage by activating genes that result in apoptosis and cell cycle arrest; (for a review see Vogelstein et al., 2000). p53 is a primary target for mutation in human cancer; it is estimated that mutations or loss of function of p53 accounts for half of all cancers. The mutation frequency of p53 varies among different tumor types so that it can be much lower than 50%, for example 30% in breast cancer and only 5% in leukemia (Soussi et al., 2005). Most p53 mutations map to the DNA binding domain. These mutants are therefore unable to activate genes upregulated by wild-type p53, a loss of function that ultimately results in unchecked cell division (Cho et al., 1994). Full-length p63 and p73, like p53, have the ability to transactivate p21 and Bax genes causing cell cycle arrest and apoptosis (Di Como et al., 1999; Gaiddon et al., 2001). Unlike p53, p63 and p73 have different isoforms (for review see Yang et al., 2002). Isoforms of p63 and p73 lacking their transactivation domains act as dominant negative inhibitors of p53 (Kaghad et al., 1997; Laan and Paabo, 1998; Yang et al., 1998). p63 and p73 knockout mice show defects in epidermal (Mills et al., 1999; Yang et al., 1999) and neuronal (Pozniak et al., 2002; Yang et al., 2000) development respectively, but do not form spontaneous tumors as is the case for p53 knockout mice (Donehower et al., 1992); the murine knockout phenotypes are mirrored in human disease. Mutations in p63 and p73 are rare in human cancer but a number of mutations in the DNA binding domain and sterile α motif of p63 are associated with genetic epidermal syndromes (van Bokhoven and McKeon, 2002). Overexpression of p73 triggers neuronal cell differentiation *in vitro* (De Laurenzi et al., 2000). There is also an important functional relationship between the

p53 family members: in the absence of p63 and p73, p53-dependent apoptosis in response to DNA damage is severely impaired (Flores et al., 2002).

[0004] The discovery of the apoptosis stimulating protein of p53 (ASPP) family of proteins revealed a new level of p53 regulation. There are three family members in humans ASPP1, ASPP2 and iASPP. ASPP1 and ASPP2 selectively stimulate the transactivation function of all three p53 family members p53, p63 and p73, increasing the transactivation of pro-apoptotic genes (Bergamaschi et al., 2004; Samuels-Lev et al., 2001). In contrast, iASPP is an oncoprotein that specifically inhibits p53; its overexpression confers resistance to apoptosis induced by UV radiation and exposure to the chemotherapeutic agent cisplatin in cultured cells (Bergamaschi et al., 2003). There is only one ASPP family member in *C.elegans* which is functionally interchangeable with human iASPP (Bergamaschi et al., 2003). Thus iASPP is likely to be the most ancient member of the ASPP family. Full length human iASPP consists of 828 amino acids, and is the isoform almost exclusively expressed in cells (Slee et al., 2004). iASPP only shares high sequence homology with ASPP1 and ASPP2 in the C-terminus region (~230 amino acids) which is made up of four ankyrin repeats and an SH3 domain, two common structural motifs involved in protein – protein interactions. In all members of the ASPP family this C-terminal region binds to the DNA binding domain of p53, and in iASPP is required for the inhibitory effect (Bergamaschi et al., 2003; Slee et al., 2004). The crystal structure of the C-terminal region of ASPP2 in complex with the DNA binding domain of p53 revealed that the ankyrin repeats and SH3 domain of ASPP2 both contribute to the p53 binding site unit (Gorina and Pavletich, 1996).

[0005] Increased expression of iASPP appears to be a mechanism involved in preventing p53 from working effectively and may be a prognostic marker for human cancers (Sullivan and Lu, 2007). It has also been suggested that targeting iASPP to reduce its expression will allow new therapeutic strategies for the treatment of cancer to be developed.

Summary of the Invention

[0006] The present inventors have crystallized the C-terminal region of inhibitory Apoptosis-Stimulating Protein of P53 (iASPP) to examine its structure in order to understand further its interaction with p53. Crystals of the C-terminal region were examined to determine the regions that interact with p53. During the course of this analysis it was also observed that the N-terminal region of the iASPP fragment occupied the p53-binding site of a neighbouring

molecule. An eight amino acid peptide (SEQ ID NO:2, referred to herein as peptide P1), based on this N-terminal region was shown to bind to iASPP.

[0007] Thus the invention relates, in its broadest aspect, to the three-dimensional structure of iASPP and its use. Particular embodiments relate to the regions of the structure that interact with p53 or with the 8 amino acid region referred to above, and to methods for the modeling and discovery of structures that mimic or compete with such binding in order to antagonize the action of iASPP on p53.

[0008] In another aspect, the invention includes an isolated compound having a spatial arrangement of atoms to bind to iASPP in a manner that interferes with iASPP binding to an iASPP binding partner selected from the group consisting of p53, p63 and p73, wherein the compound interacts with an iASPP polypeptide defined by at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 75 or more structural coordinates identified in Table 1, wherein the relative atomic positions of the structure identified in Table 1 are varied within a root mean square deviation of less than 1.2 Å. In some embodiments, the isolated compound interacts with the iASPP in a region of iASPP defined by at least ten coordinates of atoms from at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11 or at least 12 different amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2 Å.

[0009] The term “iASPP binding partner” as used herein refers to a macromolecules such proteins or protein complexes that bind to iASPP in a cell that expresses iASPP, including p53, p63 and p73. It should be understood that a fragment of a binding partner that retains iASPP binding activity is considered a binding partner for purposes of such assays.

[0010] In some embodiments, the isolated compound interacts with the iASPP in a region of iASPP defined by at least 10 coordinates of atoms from at least 10 amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2 Å. In one exemplary embodiment, the at least ten amino acids of iASPP are selected from the group consisting of T722, L724, W767, E772, D775, E776, E795, D797, W798, Y809, P811, N813 and Y814.

[0011] In some embodiments, the compound is selected from the group consisting of a small molecule, an antibody or an antigen-binding fragment thereof, a nucleic acid molecule and a peptide/polypeptide.

[0012] In some embodiments, the isolated compound is a peptide having a structure selected from the group consisting of

[0013] (a) a compound comprising a sequence of eight amino acids X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 (SEQ ID NO: 6), wherein X_1 is selected from Serine or Threonine or conservative substitution thereof; X_2 is proline or conservative substitution thereof; X_3 , X_4 , X_6 , and X_7 are each independently selected from Arginine and Lysine; X_5 is Alanine, Serine, Valine, Glycine or conservative substitutions thereof; and X_8 is Alanine, Valine, Glycine or conservative substitutions thereof;

[0014] (b) a compound with the structure of (a), with the proviso that one or more of the X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_8 amino acids are replaced with an unnatural amino acid;

[0015] (c) a compound with the structure of (a) or (b), with the proviso that one or more amide bonds is replaced with an ester or alkyl bond.

[0016] In some embodiments, the peptides described herein are from about 8 amino acid residues in length to about 100 amino acid residues in length. Of course it is contemplated that longer or indeed shorter peptides also may prove useful. Thus, peptides of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 amino acids in length are contemplated.

[0017] In a further aspect, the invention provides a compound comprises an amino acid an amino acid sequence at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at 81%, at least 82%, at least 83%, at least 84%, at least 85%, least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to a peptide/polypeptide described herein, wherein the compound retains the ability to interact (e.g., bind to) with iASPP.

[0018] In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 2, wherein the peptide retains the ability to interact (e.g., bind to) with iASPP. In some

embodiments, the compound is a peptide consisting of the amino acid sequence SPRKARRA (SEQ ID NO: 2). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 3, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRKSRRRA (SEQ ID NO: 3). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 4, wherein the peptide retains the ability to interact with (bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRRARRA (SEQ ID NO: 4). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 5, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRRSRRRA (SEQ ID NO: 5).

[0019] Cyclized versions of any of the peptides described herein are contemplated.

[0020] Nucleic acid molecules encoding a peptide/polypeptide described herein are also contemplated as another aspect of the invention. In some embodiments, the invention provides a nucleic acid molecule comprising a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to a peptide/polypeptide described herein.

[0021] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 2, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRKARRA (SEQ ID NO: 2). In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 3, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the

nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRKSRRRA (SEQ ID NO: 3). In some embodiments, the the nucleic acid molecule comprises a nucleotide sequence encoding a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to to SEQ ID NO: 4, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound comprises a nucleic acid molecule comprises a nucleotide sequence encoding a peptide consisting of the amino acid sequence SPRRARRA (SEQ ID NO: 4). In some embodiments, the compound comprises a nucleic acid molecule comprises a nucleotide sequence that encodes an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5%, or more identical to SEQ ID NO: 5, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRRSRRRA (SEQ ID NO: 5).

[0022] Any synthesis method can be used for making polypeptides and peptides of the invention. Numerous chemical synthesis methods are known and are especially suitable for shorter peptides. The isolated peptides described herein are optionally made by recombinant techniques *in vitro* and/or expressed *in vivo*. Polynucleotides that comprise nucleotide sequences that encode all (or a portion of) a peptide are an additional aspect of the invention. Vectors including expression vectors for *in vitro* production and gene therapy vectors for *in vivo* production/expression of the peptide(s), are also considered an aspect of the invention.

[0023] For example, the invention includes polynucleotides comprising a nucleotide sequence that encodes any peptide or polypeptide described herein, including an isolated peptide(s) as discussed above and described in further detail in the description below. In specific embodiments, the polynucleotide further comprises a nucleotide sequence that encodes a signal peptide fused in-frame with the polypeptides described above. The signal peptide facilitates extracellular secretion of the encoded peptide/polypeptide when expressed in a suitable host cell.

[0024] The invention also includes an expression vector comprising a nucleotide sequence that encodes any peptide (or polypeptide) described herein, including an isolated peptide(s) (or isolated polypeptide(s)) operably linked to an expression control sequence or promoter sequence. The expression vector may be any vector used for the expression of a nucleic acid and may for example, be selected from the group consisting of replication deficient adenoviral vectors, adeno-associated viral vectors, and lentivirus vectors. The polynucleotides and vectors

described herein may be formulated as compositions in which the polynucleotides or the vector is presented in a pharmaceutically acceptable carrier, excipient or diluent.

[0025] Other aspects of the invention include host cells (including isolated host cells) that have been transformed or transfected with a polynucleotide or vector of the invention. In some variations, the cells are any prokaryotic or eukaryotic cell that can be manipulated (e.g., through transformation or transfection) to express peptides or polypeptides described herein. In some variations, the cells are suitable for *ex vivo* transfection/transformation and reimplantation into a host organism.

[0026] Another aspect of the invention includes a method of making a peptide or polypeptide described herein comprising growing a host cell described herein in a growth medium under conditions where the cell expresses the peptide or polypeptide encoded by the polynucleotide. In one aspect, the peptide or polypeptide is isolated or purified from the cell or the medium. Isolation of the polypeptide from the cells or from the medium in which the cells are grown is accomplished by purification methods known in the art, *e.g.*, conventional chromatographic methods including immunoaffinity chromatography, receptor affinity chromatography, hydrophobic interaction chromatography, lectin affinity chromatography, size exclusion filtration, cation or anion exchange chromatography, high pressure liquid chromatography (HPLC), reverse phase HPLC, and the like. Still other methods of purification include those wherein the desired protein is expressed and purified as a fusion protein having a specific tag, label, or chelating moiety that is recognized by a specific binding partner or agent. The purified protein can be cleaved to yield the desired protein, or be left as an intact fusion protein. Cleavage of the fusion component may produce a form of the desired protein having additional amino acid residues as a result of the cleavage process.

[0027] Antibodies are contemplated as another aspect of the invention. In one aspect, the antibody is an antibody or antigen-binding fragment thereof that specifically binds to an iASPP epitope defined by at least 10 coordinates of atoms from at least 10 different amino acid residues identified in Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2 Å. In some embodiments, the antibody or antigen binding fragment thereof specifically binds to an epitope that includes one or more iASPP amino acids selected from the group consisting of T722, L724, W767, E772, D775, E776, E795, D797, W798, Y809, P811, N813 and Y814. The antibody or antigen binding fragment can be a monoclonal antibody.

Also contemplated are human, humanized and chimeric antibodies. A hybridoma cell line that produces the monoclonal antibody is also contemplated.

[0028] Compositions comprising a peptide, polypeptide, polynucleotide, or vector described herein and a pharmaceutically acceptable carrier are also contemplated. In certain aspects, the composition further comprises the further therapeutic or therapy. In other aspects, the further therapeutic or therapy is provided in a composition separate from the peptide, polypeptide, polynucleotide or vector.

[0029] In another aspect, the invention provides a method of treating a neoplastic disorder comprising administering to a subject in need of treatment for a neoplastic disorder a composition described herein, in an amount effective to treat the neoplastic disorder. In some embodiments, a subject is identified as having a neoplastic disorder prior to the administration of the composition. In another aspect, the invention provides a method of selecting a therapeutic regimen for a subject comprising identifying a subject as having neoplastic disorder characterized by increased expression of an iASPP polypeptide; and administering to the subject a composition as described herein.

[0030] In yet another aspect, the invention provides a method of treating a condition associated with tumor cell growth in a subject comprising administering to said subject a therapeutically effective amount of a combination therapy comprising agents selected from the group consisting of a composition as described herein and a standard of care anti-neoplastic therapy. In preferred embodiments, the agents are administered in an amount effective to inhibit tumor cell growth.

[0031] In some embodiments, the identifying step may comprise identifying a subject with a neoplastic disorder characterized by elevated expression of an iASPP polypeptide in the neoplastic cells.

[0032] Identifying steps of the methods described herein may comprise screening a biological sample suspected of containing neoplastic cells from the subject for elevated expression of the iASPP polypeptide. Exemplary biological samples suspected of containing neoplastic cells include, but are not limited to, blood, urine, spinal fluid, bone marrow, biopsy tissue, a tumor, and a sample comprising tumor cells. In some embodiments, the screening step comprises comparing expression of iASPP in cells from the sample with expression of iASPP from healthy cells of the same type as the cells in the sample, wherein increased expression of the iASPP in

cells from the sample compared to the expression of the iASPP in cells from the healthy cells indicates elevated expression of iASPP.

[0033] In some embodiments, the neoplastic disease is a cancer selected from the group consisting of breast cancer, pancreatic cancer, ovarian cancer, colorectal cancer, esophageal cancer, lung cancer, head cancer, neck cancer, gastric cancer and epithelial cancer.

[0034] Also provided is a method of stimulating p53-mediated apoptosis in a cell comprising contacting the cell with a composition described herein in amount effective to stimulate p53-mediated apoptosis. In some embodiment, the cell expresses wild-type p53.

[0035] In some aspects, and as a variation of all therapies described herein, the methods described herein comprise further administering to the subject or contacting a cell with a standard of care anti-neoplastic therapeutic. In the context of methods of the invention, “standard of care” refers to a treatment that is generally accepted by clinicians for a certain type of patient diagnosed with a type of illness. Exemplary standard of care anti-neoplastic agents include, but are not limited to, a standard of care chemotherapeutic, a standard of care radiotherapeutic, or a standard of care radiation regimen for the neoplastic disorder. For all varieties of cancers and neoplastic disorders, for example, an aspect of the invention is to improve standard of care therapy with co-therapy with agents described herein that modulate (e.g., increase or stimulate) p53-mediated apoptosis.

[0036] The composition and the further therapeutic or therapy can be administered concurrently or separately. In embodiments where the further therapeutic or therapy and composition described herein are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the further therapeutic or therapy and the composition described herein would still be able to exert an advantageously combined effect. In such instances, it is contemplated that one would administer both modalities within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations. Repeated treatments with one or both agents are specifically contemplated. Exemplary routes of administration of the peptides or compositions

described herein include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, topical, oral and intranasal administration.

[0037] In another aspect, the invention includes a method of screening for a modulator of binding of iASPP and an iASPP binding partner. An exemplary method comprises, (a) contacting an iASPP polypeptide comprising a structure defined by the structural coordinates provided in Table 1 with an iASPP binding partner, in the presence and absence of a test compound, under conditions in which the binding partner binds with the iASPP carboxy-terminal domain; wherein the binding partner comprises a member selected from the group consisting of p53, p63 and p73; and (b) comparing binding between said carboxy-terminal iASPP polypeptide and the binding partner in the presence and absence of the test compound, wherein increased binding (in the presence of the test compound, compared to the absence of the test compound) identifies the test compound as an agonist of binding, and decreased binding identifies the test compound as an antagonist inhibitor of binding.

[0038] Such assays can be performed *in vitro* in cell-free formats or cell based formats, which often lend themselves to high throughput implementation. In some cell-free variations, the iASPP or the binding partner is attached to a solid support (e.g., a bead, membrane, plate, or chip) and the other, unattached moiety is incubated with the solid support to permit binding. Optionally, the unbound moiety (the iASPP or the binding partner) is labeled (radiolabel, colorimetric label, etc.) or tagged (e.g., a peptide or epitope tag, or GFP fusion or enzymatic fusion) to facilitate measurement of binding.

[0039] In yet another aspect, the invention provides a computer-based method for the analysis of the interaction of a molecular structure with an iASPP structure. Such method comprises fitting a molecular structure to an iASPP structure defined by the structural coordinates identified in Table 1 or selected coordinates thereof, wherein the iASPP structure is optionally varied by a root square mean deviation of not more than 1.2Å, and (b) determining at least one interaction between an atom of said iASPP structure and said molecular structure.

[0040] Also provided is a computer-based method of rational drug design comprising: fitting structure of at least two molecular fragments to an iASPP structure defined by the structural coordinates identified in Table 1 or selected coordinates thereof, wherein the iASPP structure is optionally varied by a root square mean deviation of not more than 1.2Å, and assembling the fitted molecular structure fragments into a single molecule to form a single molecular structure.

[0041] In some embodiments, the selected coordinates of the iASPP structure include atoms from one or more of the amino acid residues identified in Table 3. In one variation, the selected coordinates comprise at least 5 atoms.

[0042] In some embodiments, the molecular structure comprises at least two atoms that are located in the same relative spatial orientation to each other and are of the same elements as a corresponding number of atoms found in any of the amino acids identified in Table 4 or Table 5. In some embodiments, the atoms of Table 5 include at least two atoms that contact iASPP residues via hydrogen bonds.

[0043] In some embodiments, the molecular structure is in the form of a pharmacophore.

[0044] In some embodiments, the iASPP structure is a model constructed from all or a portion of the coordinates of Table 1, optionally varied by a root mean square deviation of not more than 0.5Å. The model may be selected from the group consisting of a wire-frame model, a chicken-wire model, a ball-and-stick model, a space-filling model, a stick-model, a ribbon model, a snake model, an arrow and cylinder model, an electron density map and a molecular surface model.

[0045] In some embodiments, the computer-based methods described herein further comprise modifying the molecular structure to modulate its interaction with the iASPP structure.

[0046] In another aspect, the invention provides a computer system, intended to generate structures and/or perform optimization of compounds that interact with an iASPP C-terminal region, the system containing computer-readable data comprising the atomic coordinate data identified in Table 1 or selected coordinates thereof, optionally varied by a root mean square deviation of not more than 1.2Å. In some embodiments, the atomic coordinate data comprises at least one of the atoms provided by the amino acid residues identified in Tables 3 or 4. In some embodiments, the computer-readable data storage medium comprises data storage material encoded with said computer-readable data; a working memory for storing instructions for processing said computer-readable data; and a central-processing unit coupled to said working memory and to said computer-readable storage medium for processing said computer-readable data. In some embodiments, the computer-readable storage medium further comprises a display coupled to the central-processing unit.

[0047] In yet another aspect, the invention provides a method for providing data for generating structures and/or performing optimization of compounds which interact with an iASPP C-terminal region, the method comprises establishing communication with a remote device containing computer-readable data comprising atomic coordinate data identified in Table 1 or selected coordinate thereof, optionally varied by a root mean square deviation of not more than 1.2Å and receiving the computer readable data from the remote device. In some embodiments, the method comprises processing said computer-readable data to display a model of an iASPP C-terminal region.

[0048] Another aspect of the invention includes a computer-readable storage medium comprising a data storage material encoded with computer-readable data, wherein the data are defined by the structure identified in Table 1, optionally varied by a root mean square deviation of not more than 1.2Å.

[0049] Yet another aspect of the invention includes a computer-readable storage medium comprising a data storage material encoded with a first set of computer-readable data comprising a Fourier transform of at least a portion of the structural coordinates for the iASPP C-terminal protein defined by the structure identified in Table 1, or selected coordinates thereof, optionally varied by a root mean square deviation of not more than 1.2Å; which data, when combined with a second set of machine readable data comprising an X-ray diffraction pattern of a molecule or molecular complex or unknown structure, using a matching programmed with the instructions for using the first set of data and the second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data.

[0050] These and other aspects of the invention are set out below and in the accompanying claims. The above aspects of the invention, both singly and in combination, all contribute to features of the invention.

[0051] The foregoing summary is not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document.

[0052] In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by

specific paragraphs above. For example, certain aspects of the invention that are described as a genus, and it should be understood that every member of a genus is, individually, an aspect of the invention. Also, aspects described as a genus or selecting a member of a genus, should be understood to embrace combinations of two or more members of the genus. Although the applicant(s) invented the full scope of the invention described herein, the applicants do not intend to claim subject matter described in the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the applicant(s) by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

Brief Description of the Tables

[0053] Table 1 sets out the coordinate data of the structure of the C-terminal region of iASPP.

[0054] Table 2 sets out alterations to the P1 peptide region amino acids.

[0055] Table 3 sets out the residues of iASPP that form a peptide-binding region.

[0056] Table 4 sets out the residues of iASPP that interact with the peptide-binding region.

[0057] Table 5 sets out atoms of particular interest within Table 4 that interact with the peptide-binding region.

[0058] Table 6 provides crystallographic statistics.

Brief Description of the Drawings

[0059] Figure 1 shows saturation curves and Scatchard plots for the binding of iASPP Δ 608 and ASPP2 Δ 905 to p53_{core}, p63_{core} and p73_{core}. Each value represents the mean and standard deviation of duplicate measurements.

[0060] Figure 2 shows the overall structure of iASPP Δ 608 in (A) cartoon presentation and (B) by sequence alignment. Secondary structural features are indicated in relation to both sequences. In Figure 2(B) the symbols above the sequences represent iASPP residues observed to interact with the N-terminal residues (616-623 of SEQ ID NO: 8) of a neighbouring molecule

in the crystal lattice. The symbols below the sequences represent ASPP2 residues involved in binding to the p53 DNA binding domain in the ASPP2-p53 crystal structure (PDB code 1YCS). Arrow heads depict hydrogen bonds, squares depict hydrophobic interactions and full arrows depict residues involved in both hydrogen bonds and hydrophobic interactions.

[0061] Figure 3 shows a comparison of (A) the interaction between iASPP molecules in the crystal packing and (B) the interaction between iASPP (upper structure) and p53 (lower structure).

[0062] Figure 4 shows the interaction between the peptide P1 region and the iASPP p53-binding site of a neighboring molecule. Dotted lines represent hydrogen bonds with the lengths in Å and “eyelashes” represent hydrophobic interactions.

[0063] Figure 5 shows iASPP binds peptide P1 *in vitro*. A representative ITC experiment showing raw data (upper) and integrated data (lower) for titrations of iASPPΔ625 with the peptide P1. The dissociation constant is approximately 45 mM.

Detailed Description of the Invention

A. Protein Crystals.

[0064] The present invention provides a crystal of the iASPP C-terminal domain having an space group $P2_12_12$, and unit cell dimensions of about $a = 60.0 \text{ Å}$, $b = 67.5 \text{ Å}$, $c = 50.5 \text{ Å}$, $\alpha = \beta = \gamma = 90^\circ$, with a unit cell variability of 5% in all dimensions. An example of such variability is a crystal of unit cell dimensions $a = 59.969 \text{ Å}$, $b = 67.545 \text{ Å}$, $c = 50.490 \text{ Å}$.

[0065] Such crystals may be obtained using the methods described in the accompanying examples.

[0066] The iASPP C-terminal domain may comprise the sequence of residues 8-228 of SEQ ID NO: 1 illustrated herein, or a variant which retains the ability to form crystals under the conditions illustrated herein. Such variants include those with a number of amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids by an equivalent or fewer number of amino acids. Further examples of variants are discussed further herein below.

[0067] Generally the C-terminal domain comprising residues 8-228 of SEQ ID NO:1 will be flanked, at the N- and/or C-terminal by short sequences associated with the expression vector from which the protein is produced. Such vector sequences may include an N-terminal

methionine and short tags (such as a polyhistidine tag), e.g. of from 1 to 10 amino acids in length, to facilitate expression, recovery or purification of the sequence. For example in SEQ ID NO:1 there is an N-terminal methionine followed by six histidine residues. It is believed that the presence of from 1 to 10 amino acids at the N- and/or C-termini of the 8-228 region of SEQ ID NO:1 will not substantially alter the folding or structure of this region of the iASPP protein.

[0068] The methodology used to provide the iASPP crystal illustrated herein may be used generally to provide such crystals resolvable at a resolution of at least 2.1 Å.

[0069] The present invention contemplates “variants” wherein a “variant” refers to a polypeptide which is obtained by replacing at least one amino acid residue in iASPP of SEQ ID NO:1 with a different amino acid residue and/or by adding and/or deleting amino acid residues within the native polypeptide or at the N- and/or C-terminus of a polypeptide corresponding to iASPP. Variants may be prepared for example by site-specific mutagenesis of nucleic acid coding for the iASPP followed by expression of the iASPP in a host cell. Variants desirably have substantially the same three-dimensional structure as the iASPP from which they are derived.

[0070] By having substantially the same three-dimensional structure is meant having a set of atomic structure co-ordinates that have a root mean square deviation (rmsd) of less than or equal to about 1.2 Å when superimposed with the atomic structure co-ordinates of the iASPP of SEQ ID NO:1 from which the variant is derived.

[0071] Amino acid substitutions, deletions and additions which do not significantly interfere with the three-dimensional structure of the iASPP will depend, in part, on the region of the iASPP where the substitution, addition or deletion occurs. In highly variable regions of the molecule, non-conservative substitutions as well as conservative substitutions may be tolerated without significantly disrupting the three-dimensional structure of the molecule. In highly conserved regions, or regions containing significant secondary structure, conservative amino acid substitutions are preferred.

[0072] Conservative amino acid substitutions are well-known in the art, and include substitutions made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the amino acid residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having

similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine. Other conservative amino acid substitutions are well known in the art.

[0073] In some instances, it may be particularly advantageous or convenient to substitute, delete and/or add amino acid residues in order to provide convenient cloning sites in the cDNA encoding the polypeptide, to aid in purification of the polypeptide, etc. Such substitutions, deletions and/or additions which do not substantially alter the three dimensional structure of the iASPP will be apparent to those having skills in the art.

[0074] The residues for alteration could easily be identified by those skilled in the art and these alterations can be introduced by site-directed mutagenesis e.g. using a Stratagene QuikChangeTM Site-Directed Mutagenesis Kit or cassette mutagenesis methods (see e.g. Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, and Sambrook et al., *Molecular Cloning: a Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, (1989)).

[0075] In one aspect, crystals comprising one or more, e.g. 1 to 3 such as 1 or 2 alterations of the region corresponding to peptide P1 are contemplated. Peptide P1 corresponds to amino acids 616-623 of full-length iASPP set forth in SEQ ID NO: 8. In some embodiments, the residues of which may be altered, and the alterations contemplated, may be selected from the those set out in Table 2:

Table 2:

iASPP P1 peptide region	Alterations
SER 616	Thr
PRO 617	
ARG 618	Lys
LYS 619	Arg
ALA 620	Ser, Val, Gly
ARG 621	Lys
ARG 622	Lys
ALA 623	Val, Gly

[0076] Examples of such alterations include the sequence alterations of peptide P1 corresponding to the substitutions of SEQ ID NO:3, 4 or 5. Such crystals may be formed using

the conditions set out in the accompanying examples, and, if necessary or desirable, variations of these conditions within the routine skill and knowledge of those of ordinary skill in the art.

B. Crystal Coordinates.

[0077] In a further aspect, the invention also provides a crystal of the iASPP C-terminal region having the three dimensional atomic coordinates of Table 1. As indicated in Table 1, this provides the atomic coordinates of non-hydrogen atoms of the amino acids 616 to 822 of full-length iASPP set forth in SEQ ID NO: 8.

[0078] An advantageous feature of the structures defined by the atomic coordinates of Table 1 are that they have a resolution of about 2.1 Å.

[0079] Table 1 provides atomic coordinate data for the iASPP C-terminal region. In this Table, the first column is the identifier "ATOM", the second column a contiguous number to uniquely refer to an atom of a particular row, the third column denotes the atom, the fourth the residue type, the fifth the chain identification, the sixth the residue number (the atom numbering is with respect to the full length wild type protein), the seventh, eighth and ninth columns are the X, Y, Z coordinates respectively of the atom in question, the tenth column the occupancy of the atom, the eleventh the temperature factor of the atom, the twelfth the chain identifier.

[0080] Table 1 is set out in an internally consistent format. For example (apart from the first residue, Ser 616) the coordinates of the atoms of each amino acid residue are listed such that the backbone nitrogen atom is first, followed by the C-alpha backbone carbon atom, designated CA, followed by side chain residues (designated according to one standard convention) and finally the carbon and oxygen of the protein backbone. Alternative file formats (e.g. such as a format consistent with that of the EBI Macromolecular Structure Database (Hinxton, UK)) which may include a different ordering of these atoms, or a different designation of the side-chain residues, may be used or preferred by others of skill in the art. However it will be apparent that the use of a different file format to present or manipulate the coordinates of the Table is within the scope of the present invention.

[0081] The coordinates of Table 1 provide a measure of atomic location in Angstroms, given to 3 decimal places. The coordinates are a relative set of positions that define a shape in three dimensions, but the skilled person would understand that an entirely different set of coordinates having a different origin and/or axes could define a similar or identical shape. Furthermore, the skilled person would understand that varying the relative atomic positions of the atoms of the

structure within a root mean square deviation of less than 1.2 Å when superimposed on the coordinates provided in Table 1 will generally result in a structure which is substantially the same as the structure of Table 1 in terms of both its structural characteristics and usefulness for structure-based analysis of iASPP-interacting molecular structures.

[0082] Table 1 also sets out the position of the oxygen atom of 154 water molecules, in a similar format to the amino acid residue atoms, though with row numbering restarting from 1. Those of skill in the art will understand that changing the number and/or positions of the water molecules of the Tables will not generally affect the usefulness of the structures for structure-based analysis of iASPP-interacting structures.

[0083] Thus for the purposes described herein as being aspects of the present invention, it is within the scope of the invention if: the coordinates of any of Table 1 are transposed to a different origin and/or axes; the relative atomic positions of the atoms of the structure are varied within a root mean square deviation of less than 1.2 Å, preferably less than 1.0 Å, more preferably less than 0.5 Å, more preferably less than 0.3 Å, such as less than 0.25 Å, or less than 0.2 Å, and most preferably less than 0.1 Å, when superimposed on the coordinates provided in any of Table 1; and/or the number and/or positions of water molecules is varied.

[0084] Reference herein to the coordinate data of Table 1 and the like thus includes the coordinate data in which one or more individual values of the Table are varied in this way. By “root mean square deviation” we mean the square root of the arithmetic mean of the squares of the deviations from the mean. Reference to a root mean square deviation of less than 1.2 Å is to be interpreted as also including reference to any one of the preferred, narrower values set out above.

[0085] Protein structure similarity is routinely expressed and measured by the root mean square deviation (rmsd), which measures the difference in positioning in space between two sets of atoms, based on the square root of the arithmetic mean of the squares of the deviations from the mean. The rmsd measures distance between equivalent atoms after their optimal superposition. The rmsd can be calculated over all atoms, over residue backbone atoms (i.e. the nitrogen-carbon-carbon backbone atoms of the protein amino acid residues), main chain atoms only (i.e. the nitrogen-carbon-oxygen-carbon backbone atoms of the protein amino acid residues), side chain atoms only or more usually over C-alpha atoms only. For the purposes of

this invention, the rmsd can be calculated over any of these, using any of the methods outlined below. In one embodiment, rmsd is measured by reference to the C-alpha atoms.

[0086] Reference herein to the iASPP C-terminal structure of Table 1 and the like thus includes the iASPP C-terminal coordinate data of this Table in which one or more individual values of the Table are varied within the above-mentioned rmsd.

[0087] Methods of comparing protein structures are discussed in *Methods of Enzymology*, vol 115, pg 397-420. The necessary least-squares algebra to calculate rmsd has been given by Rossman and Argos (*J. Biol. Chem.*, vol 250, pp7525 (1975)) although faster methods have been described by Kabsch (*Acta Crystallogr., Section A*, A92, 922 (1976)); *Acta Cryst.* A34, 827-828 (1978)), Hendrickson (*Acta Crystallogr., Section A*, A35, 158 (1979)); McLachan (*J. Mol. Biol.*, vol 128, pp49 (1979)) and Kearsley (*Acta Crystallogr., Section A*, A45, 208 (1989)). Some algorithms use an iterative procedure in which the one molecule is moved relative to the other, such as that described by Ferro and Hermans (Ferro and Hermans, *Acta Crystallographic*, A33, 345-347 (1977)). Other methods e.g. Kabsch's algorithm locate the best fit directly.

[0088] Programs for determining rmsd include MNYFIT (part of a collection of programs called COMPOSER, Sutcliffe, M.J., Haneef, I., Carney, D. and Blundell, T.L. (1987) *Protein Engineering*, 1, 377-384), MAPS (Lu, G. *An Approach for Multiple Alignment of Protein Structures* (1998, in manuscript and on <http://bioinfo1.mbfys.lu.se/TOP/maps.html>)).

[0089] It is usual to consider C-alpha atoms and the rmsd can then be calculated using programs such as LSQKAB (Collaborative Computational Project 4. *The CCP4 Suite: Programs for Protein Crystallography, Acta Crystallographica*, D50, (1994), 760-763), QUANTA (Jones et al., *Acta Crystallography* A47 (1991), 110-119 and commercially available from Accelrys, San Diego, CA), Insight (commercially available from Accelrys, San Diego, CA), Sybyl® (commercially available from Tripos, Inc., St Louis), O (Jones et al., *Acta Crystallographica*, A47, (1991), 110-119), and other coordinate fitting programs.

[0090] In, for example the programs LSQKAB and O, the user can define the residues in the two proteins that are to be paired for the purpose of the calculation. Alternatively, the pairing of residues can be determined by generating a sequence alignment of the two proteins e.g. using programs for sequence alignment such as those of the BLAST suite of programs. The atomic coordinates can then be superimposed according to this alignment and an rmsd value calculated. The program Sequoia (C.M. Bruns, I. Hubatsch, M. Ridderström, B. Mannervik, and J.A. Tainer

(1999) Human Glutathione Transferase A4-4 Crystal Structures and Mutagenesis Reveal the Basis of High Catalytic Efficiency with Toxic Lipid Peroxidation Products, *Journal of Molecular Biology* 288(3): 427-439) performs the alignment of homologous protein sequences, and the superposition of homologous protein atomic coordinates. Once aligned, the rmsd can be calculated using programs detailed above. For sequence identical, or highly identical, the structural alignment of proteins can be done manually or automatically as outlined above. Another approach would be to generate a superposition of protein atomic coordinates without considering the sequence.

[0091] Those of skill in the art will appreciate that in many applications of the invention, it is not necessary to utilise all the coordinates of Table 1, but merely a portion of them. For example, as described below, in methods of modelling Table 1 structures with molecular structures, selected coordinates of the iASPP C-terminal structure of Table 1 may be used.

[0092] By “selected coordinates” it is meant for example at least 5, preferably at least 10, preferably at least 20, more preferably at least 50 and even more preferably at least 100, for example at least 500 or at least 1000 atoms of the iASPP C-terminal structure. Reference herein to selected coordinates in relation to all applications of the invention described herein is to be understood as including reference to the use of any one of these exemplified values.

[0093] In one aspect, the selected coordinates may include all or some of the atoms of the amino acid residues of Table 3. These residues are those to which the P1 peptide region of iASPP binds and are thus of interest in the development of compounds which interact with iASPP in this region in order to interfere with the binding to p53.

Table 3:

Thr 722	Leu 724	Trp 767	Glu 772
Asp 775	Glu 776	Glu 795	Asp 797
Trp 798	Tyr 809	Pro 811	Asn 813
Tyr 814			

[0094] In one aspect, the selected coordinates may comprise at least 10 coordinates (e.g. at least 20 coordinates) of atoms from at least 5 different amino acid residues of Table 3. In another aspect, the selected coordinates may comprise at least 10 coordinates (e.g. at least 20, such as at least 50) of atoms from at least 10 different residues of Table 3. References herein

below to any use of selected coordinates of Table 1 include the use of selected coordinates of Table 3, including in the above numbers and combinations.

C. Description of Structure.

[0095] In the structure of iASPP C-terminal set out in Table 1 herein, the first resolvable residue is Ser 616 of SEQ ID NO: 8 and the last residue Lys 822 of SEQ ID NO: 8. This corresponds to amino acids 16 to 222 of SEQ ID NO:1. The cDNA and amino acid sequences for full-length iASPP are set forth in SEQ ID NOs: 7 and 8, respectively.

[0096] As expected from the common domain organization of the ASPP proteins, the iASPP structure comprises four ankyrin repeats and a closely juxtaposed SH3 domain (Figure 2A) which together constitutes one structural unit with a buried surface between the ankyrin repeats and SH3 domain of 1312 Å². Ankyrin repeats 1-3 are typical in their topology, each folds into two anti-parallel α -helices followed by a β -hairpin loop which juts out at an angle of approximately 90° relative to the α -helices. The fourth repeat lacks the β -hairpin loop and has an extended second helix. The helices of one repeat pack against the helices of the adjacent repeat and the β -hairpin loops form a continuous β -sheet. The four repeats stack together forming an L-shaped structure (the stem of the L being represented by the pairs of helices and the base of the L by the β -hairpins). The inter-repeat interface mainly consists of hydrophobic interactions, and a network of hydrogen bonds connects the β -hairpin loops. There are two regions in the ankyrin repeats that contain insertions compared to the ankyrin consensus; a one residue insertion in the third ankyrin repeat located in the hairpin loop (residues 715-726 of SEQ ID NO: 8) and a second insertion of two residues between the two helices of the fourth ankyrin repeat (residues 734-742 of SEQ ID NO: 8) forming an extended loop; these features are common to ASPP proteins (Figure 2B). When compared to those in ASPP2, the iASPP ankyrin repeats (residues 627-754 of SEQ ID NO: 8) have a root mean square deviation (RMSD) of 1.2 Å for 127 equivalent C α atoms.

[0097] The ankyrin repeats are connected to the SH3 domain via the extended second helix (residues 741-754 of SEQ ID NO: 8) of the fourth ankyrin repeat which is considerably longer than the other helices of the previous repeats. Superpositions of the iASPP and ASPP2 structures considering only the SH3 domains or the ankyrin repeats, reveals a difference in the relative orientation of their two motifs of some 7 degrees, indicating that there is flexibility between these two domains. ASPP2 appears to be in a more open conformation, this is mirrored by a lower buried surface area between the ankyrin repeats and the SH3 domain (1053 Å²).

[0098] The SH3 domain (residues 758- 828 of SEQ ID NO: 8) of iASPP displays the classical β -barrel-like fold typical of SH3 domains. It comprises five anti-parallel β strands (residues 762-765 of SEQ ID NO: 8, 784-789 of SEQ ID NO: 8, 798-803 of SEQ ID NO: 8, 806-811 of SEQ ID NO: 8, 815-816 of SEQ ID NO: 8) and a 3_{10} helical segment (residues 812-814 of SEQ ID NO: 8). SH3 domain sequences have a number of highly conserved residues; the topology of an SH3 domain brings these conserved residues close together, creating a hydrophobic groove on the surface which forms a ligand binding site. Two loops which make a major contribution to this binding site have been termed the RT-loop and the n-Src loop (Figure 2). In iASPP the RT-loop is of standard length and the n-Src loop contains a three-residue insertion (residues 793-795 of SEQ ID NO: 8) of mainly acidic residues. The SH3 domain of iASPP has an RMSD of 1.0 Å when compared to that of ASPP2 for 61 equivalent C α atoms.

[0099] The ankyrin repeats and SH3 domain form inter domain hydrogen bonds between Gln 753 of SEQ ID NO: 8 in the second α -helix of the fourth ankyrin repeat and Arg 790 of SEQ ID NO: 8 positioned in the n-Src loop of the SH3 domain, and between Asn 685 in the β -hairpin loop of the second ankyrin repeat and Arg 820 of SEQ ID NO: 8 in the C-terminus of the SH3 domain. The interface is further stabilized by a network of hydrophobic interactions.

Characteristics of the p53 binding site in iASPP

[00100] The putative p53 binding site in iASPP broadly resembles that of ASPP2. The major interactions between the C-terminal region of ASPP2 and the p53 DNA binding domain, as previously reported ((Gorina and Pavletich, 1996); PDB code 1YCS), are made via the SH3 domain, which interacts with the L3 loop of p53. Further interactions are made by the β -hairpin loop of the third ankyrin repeat which interacts with the L2 loop of p53 (Gorina and Pavletich, 1996). The ASPP2 residues involved in binding the DNA binding domain of p53 are mapped onto the sequence alignment presented in Figure 2B. Many of the residues involved are conserved in iASPP but there are differences. Of the eight residues of ASPP2 that form hydrogen bonds with p53, five of these residues are identical in iASPP (Ser 725, Tyr 769, Asp 775, Glu 776 and Trp 798 of SEQ ID NO: 8) but residues Thr 722, Phe 773 and Glu 794 of SEQ ID NO: 8, differ from those at the equivalent positions in ASPP2. Of the ten ASPP2 residues observed to make hydrophobic contacts with p53, five are conserved in iASPP (Ser 725, Glu 795, Trp 798, Tyr 809 and Asn 813 of SEQ ID NO: 8). Of those that are not identical, the majority are replaced by residues with similar chemical properties except for iASPP residues Leu724, Gly 727 and Phe 773 of SEQ ID NO: 8 which respectively replace Tyr, Met and Asn in

ASPP2. Thus, although a solid phase binding assay (see Examples below) showed similar affinities for p53_{core} binding to iASPPΔ625 and ASPP2Δ905 their structures contain significant differences which could be exploited for drug design. In contrast, p63_{core} and p73_{core} binding affinities discriminate between iASPPΔ625 and ASPP2Δ905.

[00101] The explanation for this may lie in the SH3 domain. iASPP Tyr 814 is a highly conserved residue in SH3 domains, frequently involved in binding to a hydrophobic P₀ residue (most often Pro, Val or Leu) in a linear (peptide) binding motif (Wu et al., 1995; Yu et al., 1994). Comparison with the ASPP2-p53 co-structure (Gorina and Pavletich, 1996) suggests that p63 and p73 both have a Val residue at a suitable position in their L3 loops to form a favourable P₀-like interaction to Tyr 814. In the ASPP2 SH3 domain the Tyr is replaced by Leu, removing the potential for a classical P₀-like interaction, a loss consistent with the observed 3-fold reduction in binding affinity to p63_{core} and p73_{core}.

A novel interaction potential revealed by iASPP crystal packing

[00102] One unexpected property of the iASPPΔ608 crystal structure is revealed by the crystal packing. The first eight ordered N-terminal residues of one iASPPΔ608 molecule, residues 616-623 of SEQ ID NO: 8 (SPRKARRA (SEQ ID NO:2)), occupy the putative p53 binding site of the neighbouring molecule in the crystal lattice (Figure 3A, B). The majority of the interactions are made with the SH3 domain but there are also contributions from the third ankyrin repeat (Fig. 4). iASPP residues 616-623 of SEQ ID NO: 8 are orientated with the N-terminus interacting with the ankyrin repeats and the subsequent residues lying in the SH3 peptide binding groove. These residues are set out by name in Table 4.

Table 4:

SER 616	PRO 617	ARG 618	LYS 619
ALA 620	ARG 621	ARG 622	ALA 623

[00103] Pro 617 of SEQ ID NO: 8 interacts with the RT-loop and the helical segment of the SH3 domain, Arg 618 of SEQ ID NO: 8 is sandwiched between the β-hairpin loop of the third ankyrin repeat and the 3₁₀ helical segment of the SH3 domain forming hydrogen bonds to the main chain of Thr 722 of SEQ ID NO: 8. Lys 619 of SEQ ID NO: 8 interacts with the 3₁₀ helical segment and is hydrogen bonded to Glu 772 of SEQ ID NO: 8 of the RT loop of the SH3 domain; Ala 620 of SEQ ID NO: 8 is in van-der-Waals contact with the n-Src loop. Arg 622 of SEQ ID NO: 8 is coordinated by hydrogen bonds to Asp775 of SEQ ID NO: 8 and Glu 776 of

SEQ ID NO: 8 of the RT loop and Glu 795 of SEQ ID NO: 8 located on the n-Src loop and finally Ala 623 of SEQ ID NO: 8 interacts with the n-Src loop. Figure 4 details all the interactions made between the two iASPP molecules, and these are further summarized by Table 5 below.

Table 5:

Atom of Table 1	iASPP Residue	Element	Interaction*
ATOM 2	SER 616	OG	W
ATOM 5	SER 616	N	W
ATOM 8	PRO 617	CD	Ph
ATOM 11	PRO 617	CG	Ph
ATOM 13	PRO 617	O	H
ATOM 19	ARG 618	NE	W
ATOM 20	ARG 618	CZ	Ph
ATOM 21	ARG 618	NH1	H,W
ATOM 22	ARG 618	NH2	H,W
ATOM 24	ARG 618	O	H
ATOM 27	LYS 619	CB	Ph
ATOM 29	LYS 619	CD	Ph
ATOM 31	LYS 619	NZ	H
ATOM 36	ALA 620	CB	Ph
ATOM 38	ALA 620	O	H, W
ATOM 50	ARG 622	N	W
ATOM 53	ARG 622	CG	Ph
ATOM 54	ARG 622	CD	Ph
ATOM 55	ARG 622	NE	H
ATOM 56	ARG 622	CZ	Ph
ATOM 57	ARG 622	NH1	H, W
ATOM 58	ARG 622	NH2	H, W
ATOM 61	ALA 623	N	H
ATOM 63	ALA 623	CB	Ph

* W = hydrogen bond to water molecule; H = hydrogen bond to iASPP amino acid; Ph = hydrophobic interaction with iASPP amino acid.

[00104] Residues 616-623 of SEQ ID NO: 8 bury 1100 Å² of solvent accessible surface on iASPP, similar to that observed in SH3 domain-peptide co-structures (Harkiolaki et al., 2003; Wu et al., 1995). The residues bind the SH3 domain in the minus orientation (for a definition see (Feng et al., 1994; Lim et al., 1994)), interacting with a surface that shows a high level of conservation (Harkiolaki et al., 2003; Kaneko et al., 2003; Musacchio et al., 1994; Wu et al., 1995). Many of the SH3 residues involved in the interaction (Glu 776 of SEQ ID NO: 8, Trp 798 of SEQ ID NO: 8, Pro 811 of SEQ ID NO: 8, Asn 813 of SEQ ID NO: 8 and Tyr 814 of SEQ ID NO: 8) are highly conserved and mediate ligand binding in classical SH3 domain co-structures. A BLAST search (www.ncbi.nlm.nih.gov) showed that residues 616-623 of SEQ ID NO: 8 are unique to iASPP, and these residues are conserved between the human and mouse proteins.

D. Structure Solution

[00105] The atomic coordinate data of iASPP C-terminal can also be used to solve the crystal structure of other target iASPP C-terminal proteins including other crystal forms of the iASPP C-terminal region, co-complexes of the iASPP C-terminal region, where X-ray diffraction data or NMR spectroscopic data of these target iASPP C-terminal proteins has been generated and requires interpretation in order to provide a structure.

[00106] In the case of the iASPP C-terminal region, this protein may crystallize in more than one crystal form. The data of Table 1, or portions thereof, as provided by this invention, are particularly useful to solve the structure of those other crystal forms of iASPP C-terminal. It may also be used to solve the structure of iASPP variants, co-complexes, or of the crystalline form of any other protein with significant amino acid sequence homology to any functional domain of iASPP C-terminal.

[00107] In the case of other target iASPP C-terminal region proteins, this may include crystals of iASPP C-terminal region proteins which are fragments of SEQ ID NO:1 or are longer versions of iASPP, e.g. truncated nearer the N-terminal region. The present invention allows the structures of such targets to be obtained more readily where raw X-ray diffraction data is generated.

[00108] Thus, where X-ray crystallographic or NMR spectroscopic data is provided for a target iASPP C-terminal region protein of unknown three-dimensional structure, the atomic coordinate data derived from Table 1, may be used to interpret that data to provide a likely

structure for the other iASPP C-terminal region protein by techniques which are well known in the art, e.g. phasing in the case of X-ray crystallography and assisting peak assignments in NMR spectra.

[00109] One method that may be employed for these purposes is molecular replacement. In this method, the unknown crystal structure, whether it is another crystal form of an iASPP C-terminal region protein, a iASPP C-terminal variant or an iASPP C-terminal co-complex, or the crystal of a target iASPP C-terminal protein with amino acid sequence homology to any functional domain of iASPP C-terminal, may be determined using the iASPP C-terminal structure coordinates of all or part of Table 1 of this invention. This method will provide an accurate structural form for the unknown crystal more quickly and efficiently than attempting to determine such information *ab initio*.

[00110] Examples of computer programs known in the art for performing molecular replacement are CNX (Brunger A.T.; Adams P.D.; Rice L.M., Current Opinion in Structural Biology, Volume 8, Issue 5, October 1998, Pages 606-611 (also commercially available from Accelrys San Diego, CA), MOLREP (A.Vagin, A.Teplyakov, MOLREP: an automated program for molecular replacement, J. Appl. Cryst. (1997) 30, 1022-1025, part of the CCP4 suite) or AMoRe (Navaza, J. (1994). AMoRe: an automated package for molecular replacement. Acta Cryst. A50, 157-163).

[00111] Thus, in a further aspect of the invention provides a method for determining the structure of a protein, which method comprises;

[00112] providing the coordinates (or selected coordinates thereof) of the iASPP C-terminal structure of Table 1,

[00113] positioning the coordinates in the crystal unit cell of said protein so as to provide a structure for said protein.

[00114] The invention may also be used to assign peaks of NMR spectra of such proteins, by manipulation of the data of Table 1.

E. Computer Systems.

[00115] In another aspect, the present invention provides systems, particularly a computer system intended to generate structures and/or perform optimization of compounds which interact

with an iASPP C-terminal region, the system containing computer-readable data comprising atomic coordinate data of Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof.

[00116] For example the computer system may comprise: (i) a computer-readable data storage medium comprising data storage material encoded with the computer-readable data; (ii) a working memory for storing instructions for processing said computer-readable data; and (iii) a central-processing unit coupled to said working memory and to said computer-readable data storage medium for processing said computer-readable data and thereby generating structures and/or performing rational drug design. The computer system may further comprise a display coupled to said central-processing unit for displaying said structures.

[00117] The structure of the iASPP region provided by Table 1, or selected coordinates thereof, may be displayed by the computer system in the form of a model, for example (a) a wire-frame model; (b) a chicken-wire model; (c) a ball-and-stick model; (d) a space-filling model; (e) a stick-model; (f) a ribbon model; (g) a snake model; (h) an arrow and cylinder model; (i) an electron density map; (j) a molecular surface model. Such representations will be useful in the practice of various aspects of the invention described further herein.

[00118] The invention also provides such systems containing atomic coordinate data of target iASPP C-terminal proteins wherein such data has been generated according to the methods of the invention described herein based on the starting data provided the data of Table 1 or selected coordinates thereof.

[00119] Such data is useful for a number of purposes, including performing rational drug design of compounds that interact with the iASPP C-terminal region, such as compounds which are potential modulators of the interaction of iASPP with p53, p63 or p73, e.g. inhibitors of these interactions that promote apoptosis.

[00120] In a further aspect, the present invention provides computer-readable storage medium, comprising a data storage material encoded with computer readable data, wherein the data are defined by the structure of Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å.

[00121] As used herein, "computer readable media" refers to any medium or media, which can be read and accessed directly by a computer. Such media include, but are not limited to:

magnetic storage media such as floppy discs, hard disc storage medium and magnetic tape; optical storage media such as optical discs or CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

[00122] By providing such computer readable media, the atomic coordinate data of the invention can be routinely accessed to model iASPP C-terminal regions or selected coordinates thereof. For example, RASMOL (Sayle et al., *TIBS*, Vol. 20, (1995), 374) is a publicly available computer software package, which allows access and analysis of atomic coordinate data for structure determination and/or rational drug design.

[00123] As used herein, "a computer system" refers to the hardware means, software means and data storage means used to analyse the atomic coordinate data of the invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means and data storage means. Desirably a monitor is provided to visualize structure data. The data storage means may be RAM or means for accessing computer readable media of the invention. Examples of such systems are microcomputer workstations available from Silicon Graphics Incorporated and Sun Microsystems running Unix based, Windows XP or IBM OS/2 operating systems.

[00124] The invention also provides a computer-readable data storage medium comprising a data storage material encoded with a first set of computer-readable data comprising the iASPP C-terminal coordinates of Table 1 or selected coordinates thereof; which, when combined with a second set of machine readable data comprising an X-ray diffraction pattern of a molecule or molecular complex of unknown structure, using a machine programmed with the instructions for using said first set of data and said second set of data, can determine at least a portion of the electron density or structure coordinates corresponding to the second set of machine readable data.

[00125] A further aspect of the invention provides a method of providing data for generating structures and/or performing optimisation of compounds which interact with an iASPP C-terminal region, the method comprising:

[00126] (i) establishing communication with a remote device containing computer-readable data comprising atomic coordinate data of Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof; and

[00127] (ii) receiving said computer-readable data from said remote device.

[00128] When such data is received it may be utilised in the various methods of the invention described herein, including computer-based methods of the analysis of the interaction of a molecular structure with an iASPP structure. The atomic coordinate data may include coordinates of amino acids set out in Table 3 and/or Table 4.

[00129] Thus the remote device may comprise e.g. a computer system or computer readable media of one of the previous aspects of the invention. The device may be in a different country or jurisdiction from where the computer-readable data is received.

[00130] The communication may be via the internet, intranet, e-mail etc, transmitted through wires or by wireless means such as by terrestrial radio or by satellite. Typically the communication will be electronic in nature, but some or all of the communication pathway may be optical, for example, over optical fibers.

F Uses of the Structure.

Summary

[00131] The crystal structures obtained according to the present invention, as well as the structures of target iASPP C-terminal proteins obtained in accordance with the methods described herein, may be used in several ways for drug design. In one aspect, the discovery of a region of the iASPP that can be bound by a short peptide allows a process of rational drug discovery based on the targeting of interactions that replicate at least some of the P1 peptide interactions.

[00132] In the case of the P1 peptide itself (SEQ ID NO:2), or its variants including SEQ ID NOs:3-5, information on the binding orientation by either co-crystallization, soaking or computationally docking such peptides can be used to guide specific modifications to the chemical structure designed to improve the interaction of the peptide with iASPP, or to improve the size or pharmacological properties of the peptide.

[00133] Thus, the determination of the three-dimensional structure of iASPP C-terminal provides a basis for the design of new compounds, which interact with iASPP C-terminal in novel ways. For example, knowing the three-dimensional structure of iASPP C-terminal, computer modelling programs may be used to design different molecules expected to interact with possible or confirmed active sites, such as binding sites or other structural or functional

features of iASPP C-terminal. These and further aspects of the invention are described further below:

(i) Computer based methods of analysis

[00134] Although the invention will facilitate the determination of actual crystal structures comprising an iASPP C-terminal region and a compound which interacts with the iASPP C-terminal, current computational techniques provide a powerful alternative to the need to generate such crystals and generate and analyze diffraction data. Accordingly, a particularly preferred aspect of the invention relates to computer-based (sometimes referred to as “*in silico*”) methods directed to the analysis and development of compounds which interact with iASPP C-terminal structures of the present invention.

[00135] Determination of the three-dimensional structure of iASPP C-terminal provides important information about the binding sites of iASPP C-terminal, particularly when comparisons are made with similar proteins. This information may then be used for rational design and modification of iASPP C-terminal inhibitors, e.g. by computational techniques which identify possible binding ligands for the binding sites, by enabling linked-fragment approaches to drug design, and by enabling the identification and location of bound ligands using X-ray crystallographic analysis. These techniques are discussed in more detail below.

[00136] Thus as a result of the determination of the iASPP C-terminal three-dimensional structure, more purely computational techniques for rational drug design may also be used to design structures whose interaction with iASPP C-terminal is better understood (for an overview of these techniques see e.g. Walters et al (*Drug Discovery Today*, Vol.3, No.4, (1998), 160-178; Abagyan, R.; Totrov, M. *Curr. Opin. Chem. Biol.* **2001**, 5, 375-382). For example, automated ligand-receptor docking programs (discussed e.g. by Jones et al. in *Current Opinion in Biotechnology*, Vol.6, (1995), 652-656 and Halperin, I.; Ma, B.; Wolfson, H.; Nussinov, R. *Proteins* **2002**, 47, 409-443), which require accurate information on the atomic coordinates of target receptors may be used.

[00137] The aspects of the invention described herein which utilize the iASPP C-terminal structure *in silico* may be equally applied to both the iASPP C-terminal structure of Table 1 or selected coordinates thereof and the models of target iASPP C-terminal proteins obtained by other aspects of the invention. Thus having determined a conformation of a iASPP C-terminal by the method described above, such a conformation may be used in a computer-based method

of rational drug design as described herein. In addition the availability of the structure of the iASPP C-terminal will allow the generation of highly predictive pharmacophore models for virtual library screening or compound design.

[00138] Accordingly, the invention provides a computer-based method for the analysis of the interaction of a molecular structure with an iASPP structure, which comprises:

[00139] providing a structure comprising a three-dimensional representation of the C-terminal region of an iASPP structure, said structure being as set out in Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof;

[00140] providing a molecular structure to be fitted to said iASPP structure or selected coordinates thereof;

[00141] fitting the molecular structure to said iASPP structure.

[00142] By “fitting”, it is meant determining at least one interaction between an atom of said iASPP structure and said molecular structure.

[00143] Interactions may be determined by automatic, semi-automatic or manual means. Computer programs can be employed to estimate interactions including the attraction, repulsion, and steric hindrance of the two binding partners (i.e. the iASPP C-terminal and a molecular structure). Various computer-based methods for fitting are described further herein. Generally the process involves calculating the extent to which such an interaction is present or stable.

[00144] In one embodiment, the iASPP C-terminal structure described herein comprises the coordinate data set forth in Table 1, or selected coordinates thereof. In practice, it will be desirable to model a sufficient number of atoms of the iASPP C-terminal as defined by the coordinates of Table 1 or selected coordinates thereof), which represent a region of interest. In the present invention, we have identified that the region of iASPP corresponding to the P1 peptide appears to bind to the p53 binding region of the protein. The residues to which the P1 peptide bind include those set out in Table 3 above. Thus methods of the invention, including those relating to the analysis of the interaction of a molecular structure with an iASPP structure and to methods of rational drug design may utilize selected coordinates which include some or all of those of the amino acid residues of Table 3.

[00145] Although every different compound that may bind to the iASPP C-terminal region may interact with different parts of this protein, the structure of this iASPP C-terminal allows the identification of a number of particular sites which are likely to be involved in many of the interactions of iASPP C-terminal with a drug candidate, in particular the residues set out in Table 3. Thus, in this aspect of the invention, the selected coordinates may comprise coordinates of at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, or all of the residues set forth in Table 3.

[00146] In order to provide a three-dimensional structure of compounds to be fitted to a iASPP C-terminal structure of the invention, the compound structure may be modeled in three dimensions using commercially available software for this purpose or, if its crystal structure is available, the coordinates of the structure may be used to provide a representation of the compound for fitting to a iASPP C-terminal structure of the invention.

[00147] Newly designed structures may be synthesized and their interaction with iASPP C-terminal may be determined or predicted as to how the newly designed structure interacts with said iASPP C-terminal structure. This process may be iterated so as to further alter the interaction between it and the iASPP C-terminal.

[00148] More specifically, the interaction of a compound or compounds with iASPP C-terminal can be examined through the use of computer modeling using a docking program such as GOLD (Jones et al., *J. Mol. Biol.*, 245, 43-53 (1995), Jones et al., *J. Mol. Biol.*, 267, 727-748 (1997)), GRAMM (Vakser, I.A., *Proteins*, Suppl., 1:226-230 (1997)), DOCK (Kuntz et al, *J.Mol.Biol.* **1982**, 161, 269-288, Makino et al, *J.Comput.Chem.* **1997**, 18, 1812-1825), AUTODOCK (Goodsell et al, *Proteins* **1990**, 8, 195-202, Morris et al, *J.Comput.Chem.* **1998**, 19, 1639-1662.), FlexX, (Rarey et al, *J.Mol.Biol.* **1996**, 261, 470-489) or ICM (Abagyan et al, *J.Comput.Chem.* **1994**, 15, 488-506). This procedure can include computer fitting of compounds to the iASPP C-terminal to ascertain how well the shape and the chemical structure of the compound will bind to the iASPP C-terminal.

[00149] Also computer-assisted, manual examination of the active site structure of iASPP C-terminal may be performed. The use of programs such as GRID (Goodford, *J. Med. Chem.*, 28, (1985), 849-857) - a program that determines probable interaction sites between molecules with various functional groups and an enzyme surface - may also be used to predict, for example, the types of modifications which will alter the binding of a compound.

[00150] Detailed structural information can then be obtained about the binding of the compound to iASPP C-terminal, and in the light of this information adjustments can be made to the structure or functionality of the compound, e.g. to alter its interaction with iASPP C-terminal. The above steps may be repeated and re-repeated as necessary.

[00151] Molecular structures, which may be used in the present invention, will usually be compounds under development for pharmaceutical use or pharmacophore structures representative of a molecular framework that carries the essential features responsible for a drug's biological activity. Generally such compounds will be organic molecules, which are typically from about 100 to 2000 Da, more preferably from about 100 to 1000 Da in molecular weight. Such compounds include peptides and derivatives thereof, anti-cancer agents, and the like. In principle, any compound under development in the field of pharmacy can be used in the present invention in order to facilitate its development or to allow further rational drug design to improve its properties.

(ii) Fragment linking and growing.

[00152] The provision of the crystal structures of the invention will also allow the development of compounds which interact with the P1 peptide binding region of iASPP based on a fragment linking or fragment growing approach.

[00153] For example, the binding of one or more molecular fragments can be determined in the protein binding pocket by X-ray crystallography. Molecular fragments are typically compounds with a molecular weight between 100 and 200 Da (Carr et al, 2002). This can then provide a starting point for medicinal chemistry to optimize the interactions using a structure-based approach. The fragments can be combined onto a template or used as the starting point for 'growing out' an inhibitor into other pockets of the protein (Blundell et al, 2002). The fragments can be positioned in the binding pocket of the iASPP C-terminal and then 'grown' to fill the space available, exploring the electrostatic, van der Waals or hydrogen-bonding interactions that are involved in molecular recognition. The potency of the original weakly binding fragment thus can be rapidly improved using iterative structure-based chemical synthesis.

[00154] At one or more stages in the fragment growing approach, the compound may be synthesized and tested in a biological system for its activity. This can be used to guide the further growing out of the fragment.

[00155] Where two fragment-binding regions are identified, a linked fragment approach may be based upon attempting to link the two fragments directly, or growing one or both fragments in the manner described above in order to obtain a larger, linked structure, which may have the desired properties.

[00156] Where the binding site of two or more ligands are determined they may be connected to form a potential lead compound that can be further refined using e.g. the iterative technique of Greer et al. For a virtual linked-fragment approach see Verlinde et al., *J. of Computer-Aided Molecular Design*, 6, (1992), 131-147, and for NMR and X-ray approaches see Shuker et al., *Science*, 274, (1996), 1531-1534 and Stout et al., *Structure*, 6, (1998), 839-848. The use of these approaches to design iASPP C-terminal inhibitors is made possible by the determination of the iASPP C-terminal structure.

[00157] Accordingly, the present invention also provides a computer-based method of rational drug design comprising:

[00158] providing a structure comprising a three-dimensional representation of the C-terminal region of an iASPP structure, said structure being as set out in Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof;

[00159] providing the structures of a plurality of molecular fragments;

[00160] fitting the structure of at least two of the molecular fragments to the selected coordinates, wherein said fitting includes determining at least one interaction between an atom of said iASPP structure and a molecular fragment; and

[00161] assembling the fitted molecular fragments into a single molecule to form a single molecular structure.

(iii) Molecular structures and fragments.

[00162] In one aspect of the invention, molecular structures and fragments that are used in soaking, co-crystallization or computer-based methods of design may be based upon the detailed analysis provided herein on the P1 peptide and its interactions with the C-terminal region of iASPP.

[00163] Thus the methods of the invention may utilize said molecular structures or fragments that comprise at least two atoms that are located in the same relative spatial orientation to each

other and are of the same elements as a corresponding number of atoms found in any of the amino acids selected from Table 4.

[00164] In particular, the atoms may be selected from the atoms of Table 5. As noted above, Table 5 identifies atoms which interact with iASPP via hydrogen bonds or hydrophobic interactions, as well as with water molecules associated with iASPP. The former two types of interactions are particularly preferred, in particular atoms that contact iASPP residues via hydrogen bonds are of interest.

[00165] For example, molecular structures or fragments may be based upon peptide variants of the P1 peptide, including the peptides of SEQ ID NOs:3-5, or fragments thereof. Mimetics comprising a modified amide bond but retaining two or more side-chain residues which contain atoms of the same element and in the same relative orientation in space as atoms of Table 4 residues, and preferably of Table 5 atoms are also contemplated.

[00166] Thus, one aspect the invention includes a process comprising selecting two atoms of residues of Table 4, preferably atoms of Table 5; calculating the distance between said atoms; screening a library of compound structures to identify compounds comprising two atoms of the same elements as the selected atoms that are an equivalent distance apart; and fitting said compounds to the C-terminal region of an iASPP structure, said structure being as set out in Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof.

[00167] The above process may be refined by screening compound structures for three or more atoms in the same spatial orientation as the same three elements of the P1 peptide. Such a process may comprise selecting two atoms of residues of Table 4, preferably atoms of Table 5; calculating the distance between said atoms; screening a library of compound structures to identify compounds comprising two atoms of the same elements as the selected atoms that are an equivalent distance apart; further selecting those compounds which comprise a third atom of an element which is located in an equivalent relative spatial orientation to the first two atoms as an atom of the same element in a Table 4 residue; and fitting said compound to the C-terminal region of an iASPP structure, said structure being as set out in Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof.

(iv) *Analysis and modification of compounds bound to iASPP*

[00168] In any event, where a molecular structure or fragment is fitted to the C-terminal region of iASPP, e.g. by a method of the invention described herein, it will also be possible to modify the structure to modify, e.g. decrease or increase, its interaction with iASPP.

[00169] The present invention also provides a method which comprises:

[00170] fitting a starting compound to a structure comprising a three-dimensional representation of the C-terminal region of an iASPP structure, said structure being as set out in Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof;

[00171] determining at least one interaction between an atom of said iASPP structure and said compound; and

[00172] modifying the structure of the compound so as to alter the interaction between it and the iASPP structure.

[00173] It would be understood by those of skill in the art that modification of the structure will usually occur *in silico*, allowing predictions to be made as to how the modified structure interacts with the iASPP C-terminal.

[00174] Greer *et al.* (*J. of Medicinal Chemistry*, Vol. 37, (1994), 1035-1054) describes an iterative approach to ligand design based on repeated sequences of computer modeling, protein-ligand complex formation and X-ray crystallographic or NMR spectroscopic analysis. Thus novel thymidylate synthase inhibitor series were designed *de novo* by Greer *et al.*, and iASPP C-terminal ligands may also be designed or modified in the this way. More specifically, using e.g. GRID on the solved structure of iASPP C-terminal, a ligand for iASPP C-terminal may be designed that complements the functionalities of the iASPP C-terminal binding sites.

Alternatively a ligand for iASPP C-terminal may be modified such that it complements the functionalities of the iASPP C-terminal binding sites better or less well. The ligand can then be synthesised, formed into a complex with iASPP C-terminal, and the complex then analyzed by X-ray crystallography to identify the actual position of the bound ligand. The structure and/or functional groups of the ligand can then be adjusted, if necessary, in view of the results of the X-ray analysis, and the synthesis and analysis sequence repeated until an optimized ligand is obtained. Related approaches to structure-based drug design are also discussed in Bohacek *et*

al., Medicinal Research Reviews, Vol.16, (1996), 3-50. Design of a compound with alternative iASPP C-terminal properties using structure based drug design may also take into account the requirements for high affinity to a second, target protein. Gschwend *et al.*, (Bioorganic & Medicinal Chemistry Letters, Vol 9, (1999), 307-312) and Bayley *et al.*, (Proteins: Structure, Function and Genetics, Vol 29, (1997) 29-67) describe approaches where structure based drug design is used to reduce affinity to one protein whilst maintaining affinity for a target protein.

[00175] Modification will be those conventional in the art known to the skilled medicinal chemist, and will include, for example, substitutions or removal of groups containing residues which interact with the amino acid side chain groups of an iASPP C-terminal structure described herein. For example, the replacements may include the addition or removal of groups in order to decrease or increase the charge of a group in a test compound, the replacement of a group to increase or decrease the size of the group in a test compound, the replacement of a charge group with a group of the opposite charge, or the replacement of a hydrophobic group with a hydrophilic group or vice versa. It will be understood that these are only examples of the type of substitutions considered by medicinal chemists in the development of new pharmaceutical compounds and other modifications may be made, depending upon the nature of the starting compound and its activity.

[00176] Where a potential modified compound has been developed by fitting a starting compound to the iASPP C-terminal structure disclosed herein and predicting from this a modified compound with an altered property, the invention further includes the step of synthesizing the modified compound and testing it in a *in vivo* or *in vitro* biological system in order to determine its activity.

[00177] The above-described processes of the invention may be iterated in that the modified compound may itself be the basis for further compound design.

[00178] The various computer-based methods of analysis may be performed using computer systems such as those described in the preceding section. Generally, the computer systems used will be configured to display or transmit a model of the structure of Table 1, or selected coordinates thereof and a molecular structure so as to indicate one or more interactions between the two. A variety of formats of display are known in the art and may be selected by a person of ordinary skill in the art dependent upon a variety of factors including, for example, the nature of the interactions being determined.

[00179] Once a molecular structure has been obtained via the computer-based methods of analysis of the present invention, a compound may be obtained or synthesized for further testing or experimental verification.

[00180] Thus in one aspect the invention provides, following the computer-based methods of the invention described above, the steps of:

[00181] obtaining or synthesizing a compound which has said molecular structure; and

[00182] contacting said compound with an iASPP protein to determine the ability of said compound to interact with the iASPP.

[00183] In another aspect the invention provides, following the computer-based methods disclosed herein, the steps of:

[00184] obtaining or synthesizing a compound which has said molecular structure;

[00185] forming a complex of an iASPP C-terminal protein and said compound; and

[00186] analyzing said complex by X-ray crystallography to determine the ability of said compound to interact with the iASPP C-terminal region.

[00187] In a further aspect the invention provides, following the computer-based methods disclosed herein, the steps of:

[00188] obtaining or synthesizing a compound which has said molecular structure; and

[00189] determining or predicting how said compound is bound to said iASPP C-terminal structure; and

[00190] modifying the compound structure so as to alter the interaction between it and the iASPP C-terminal region.

[00191] For the avoidance of doubt, the term “modifying” is used as defined above.

[00192] The ability of a compound to interact with an iASPP protein or C-terminal region thereof may be determined experimentally. For example, the accompanying example demonstrates that iASPP binds to peptide P1 *in vitro*. A compound obtained or selected according to the present invention may be used in the method described in place of the P1

peptide or in competition with the peptide to determine if the compound can bind to the P1 peptide-binding region with a greater or similar affinity to P1.

[00193] The compound may also be tested in a culture of mammalian, e.g., human, cells to determine whether or not it enhances p53-mediated apoptosis. Such a method will generally comprise the steps of:

[00194] bringing the compound into contact with a mammalian cell culture; and

[00195] comparing the rate of apoptosis in the presence of the compound compared to its absence.

[00196] The cells may be a tumor cell line. In one embodiment the tumor cell line is be a wild-type p53 tumor cell line.

(v) Obtaining and analysing crystal complexes.

[00197] In one approach, the structure of a compound bound to an iASPP C-terminal region may be determined by experiment. This will provide a starting point in the analysis of the compound bound to iASPP, thus providing those of skill in the art with a detailed insight as to how that particular compound interacts with iASPP C-terminal region.

[00198] Many of the techniques and approaches to structure-based drug design described above rely at some stage on X-ray analysis to identify the binding position of a ligand in a ligand-protein complex. A common way of doing this is to perform X-ray crystallography on the complex, produce a difference Fourier electron density map, and associate a particular pattern of electron density with the ligand. However, in order to produce the map (as explained e.g. by Blundell et al., in *Protein Crystallography*, Academic Press, New York, London and San Francisco, (1976)), it is necessary to know beforehand the protein 3D structure (or at least the protein structure factors). Therefore, determination of the iASPP C-terminal structure also allows difference Fourier electron density maps of iASPP C-terminal-compound complexes to be produced, determination of the binding position of the drug and hence may greatly assist the process of rational drug design.

[00199] Accordingly, the invention provides a method for determining the structure of a compound bound to an iASPP C-terminal region, said method comprising:

[00200] providing a crystal of an iASPP C-terminal region;

[00201] soaking the crystal with the compound to form a complex; and

[00202] determining the structure of the complex by employing the data of Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof.

[00203] Alternatively, the iASPP C-terminal and compound may be co-crystallized. Thus the invention provides a method for determining the structure of a compound bound to an iASPP C-terminal region, said method comprising:

[00204] mixing iASPP C-terminal protein with the compound;

[00205] crystallizing a iASPP C-terminal protein-compound complex; and

[00206] determining the structure of the complex by employing the data of Table 1 optionally varied by a root mean square deviation of not more than 1.2 Å, or selected coordinates thereof.

[00207] In both approaches, the iASPP C-terminal protein may be that of SEQ ID NO: 1 (which corresponds to residues 608-828 of full-length iASPP, Genbank Accession No. NP_006654) or may be an iASPP C-terminal region protein which is a fragment of SEQ ID NO:1, or is a longer version of iASPP, e.g. truncated nearer the N-terminal region.

[00208] In some aspects, a mixture of compounds may be used for soaking or co-crystallization. For example, a diverse mixture of compounds representing pharmacophore structures may be employed to determine which member or members of the mixture bind to iASPP. Where two or more components of the mixture are found to bind, these may be used as the basis for fragment linking analysis such as that described below.

[00209] The analysis of the co-crystal structures obtained by soaking or co-crystallization may employ (i) X-ray crystallographic diffraction data from the complex and (ii) a three-dimensional structure of iASPP C-terminal, or at least selected coordinates thereof, to generate a difference Fourier electron density map of the complex, the three-dimensional structure being defined by atomic coordinate data of Table 1 or selected coordinates thereof. The difference Fourier electron density map may then be analyzed.

[00210] Therefore, such complexes can be crystallized and analyzed using X-ray diffraction methods, e.g. according to the approach described by Greer et al., *J. of Medicinal Chemistry*, Vol. 37, (1994), 1035-1054, and difference Fourier electron density maps can be calculated based on X-ray diffraction patterns of soaked or co-crystallized protein and the solved structure of uncomplexed protein. These maps can then be analyzed e.g. to determine whether and where a particular compound binds to the iASPP C-terminal region and/or changes the conformation of this region.

[00211] Electron density maps can be calculated using programs such as those from the CCP4 computing package (Collaborative Computational Project 4. The CCP4 Suite: Programs for Protein Crystallography, *Acta Crystallographica*, D50, (1994), 760-763.). For map visualization and model building programs such as "O" (Jones et al., *Acta Crystallographica*, A47, (1991), 110-119) can be used.

[00212] In addition, iASPP C-terminal variants may be crystallized in co-complex with known iASPP C-terminal substrates or inhibitors or novel compounds, such as peptide P1. The crystal structures of a series of such complexes may then be solved by molecular replacement and compared with that of the iASPP C-terminal structure of Table 1 or selected coordinates thereof. This information provides an additional tool for determining the most efficient binding interactions, for example, increased hydrophobic interactions, between the iASPP C-terminal region and a chemical entity or compound.

[00213] All of the complexes referred to above may be studied using well-known X-ray diffraction techniques and may be refined against 1.5 to 3.5 Å resolution X-ray data to an R value of about 0.30 or less using computer software, such as CNX (Brunger et al., *Current Opinion in Structural Biology*, Vol. 8, Issue 5, October 1998, 606-611, and commercially available from Accelrys, San Diego, CA), and as described by Blundell et al, (1976) and *Methods in Enzymology*, vol. 114 & 115, H. W. Wyckoff et al., eds., Academic Press (1985).

[00214] This information may thus be used to optimize molecular structures that bind to the iASPP C-terminal region, and more importantly, to design and synthesize novel classes of such structures.

[00215] Information obtained on complexes of iASPP and a compound may be used to initiate or optimize the computer-based methods described herein.

(vi) *Compounds of the invention.*

[00216] A therapy based on the inhibition of iASPP binding p53 - or either of p63 and p73 - could be a target for cancer therapy (Bergamaschi et al., 2003; Trigiante and Lu, 2006). SH3 domains have proved to be difficult targets for drug design as most SH3 ligand interactions show high cross-reactivity with several SH3 domains (Feng et al., 1994; Feng et al., 1995; Knudsen et al., 1995; Lim et al., 1994; Wu et al., 1995). The uniqueness of the p53 binding site, made up of contributions from both the SH3 domain and the third ankyrin may provide a druggable target. Protein-protein interactions observed in the iASPP crystal structure provide a starting point for structure based drug design. For example, residues Arg 618 of SEQ ID NO: 8 and Lys 619 of SEQ ID NO: 8, from a neighbouring molecule in the crystal lattice, interact with residues unique to iASPP (not present in ASPP1 or ASPP2) such as Thr 722 of SEQ ID NO: 8 and Leu 724 of SEQ ID NO: 8. A small molecule that mimicked this binding mode could specifically block the p53 binding site of iASPP and as such provide a novel strategy for inhibition of this oncoprotein.

[00217] Thus where a compound has been developed by fitting a starting compound to the iASPP C-terminal structure of the invention and obtaining from this a modified compound the invention further includes the step of synthesizing the modified compound and testing it in an *in vivo* or *in vitro* biological system in order to determine its activity, e.g. in binding to iASPP or modifying apoptosis in a cell.

[00218] In another aspect, the invention includes a compound, which is identified by the methods of the invention described above.

[00219] In another aspect, the invention includes an isolated compound having a spatial arrangement of atoms to bind to iASPP in a manner that interferes with iASPP binding to an iASPP binding partner selected from the group consisting of p53, p63 and p73, wherein the compound interacts with an iASPP polypeptide defined by at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 75 or more structural coordinates identified in Table 1, wherein the relative atomic positions of the structure identified in Table 1 are varied within a root mean square deviation of less than 1.2Å. In some embodiments, the isolated compound interacts with the iASPP in a region of iASPP defined by at least ten coordinates of atoms from at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11 or at least 12 different amino acids of Table 3,

wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2 Å.

[00220] In some embodiments, the isolated compound interacts with the iASPP in a region of iASPP defined by at least 10 coordinates of atoms from at least 10 amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2 Å. In one exemplary embodiment, the at least ten amino acids of iASPP are selected from the group consisting of T722, L724, W767, E772, D775, E776, E795, D797, W798, Y809, P811, N813 and Y814.

[00221] In some embodiments, the compound is selected from the group consisting of a small molecule, an antibody or an antigen-binding fragment thereof, a nucleic acid molecule and a polypeptide.

[00222] In some embodiments, the isolated compound has a structure selected from the group consisting of

[00223] (a) a compound comprising a sequence of eight amino acids X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈ (SEQ ID NO: 6), wherein X₁ is selected from Serine or Threonine or conservative substitution thereof; X₂ is proline or conservative substitution thereof; X₃, X₄, X₆, and X₇ are each independently selected from Arginine and Lysine; X₅ is Alanine, Serine, Valine, Glycine or conservative substitutions thereof; and X₈ is Alanine, Valine, Glycine or conservative substitutions thereof;

[00224] (b) a compound with the structure of (a), with the proviso that one or more of the X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈ amino acids are replaced with an unnatural amino acid;

[00225] (c) a compound with the structure of (a) or (b), with the proviso that one or more amide bonds is replaced with an ester or alkyl bond.

[00226] In some embodiments, the amino acid sequence set forth in SEQ ID NO: 6 includes the amino acids set forth below in Table 6 below.

X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈
Serine	Proline	Arginine	Arginine	Alanine	Arginine	Arginine	Alanine
Threonine		Lysine	Lysine	Serine	Lysine	Lysine	Valine
	3-Hydroxy-	Hydroxy-lysine	Hydroxy-lysine	Glycine	Hydroxy-lysine	Hydroxy-lysine	Glycine

	proline						
	4-Hydroxy-proline			N-ethyl-glycine			N-ethyl-glycine
				N-methyl-glycine			N-methyl-glycine
				β -alanine			N-methyl-valine
							Norvaline
							β -alanine

[00227] Any amino acid identified as X₁ in Table 6 above can be paired with any amino acid identified as X₂, X₃, X₄, X₅, X₆, X₇ or X₈ in Table 6, for a total of 23,382 individual peptides. All individual peptides are contemplated as aspects of the invention.

[00228] In another embodiment, the compound is a peptide comprising a sequence of eight amino acids X₁-X₂-X₃-X₄-X₅-X₆-X₇-X₈ (SEQ ID NO: 6), wherein

[00229] X₁ is selected from Serine or Threonine or a conservative substitution thereof;

[00230] X₂ is Proline or a conservative substitution thereof;

[00231] X₃, X₄, X₆, and X₇ are each independently selected from Arginine and Lysine or a conservative substitution thereof;

[00232] X₅ is Alanine, Serine, Valine or Glycine or a conservative substitution thereof; and

[00233] X₈ is Alanine, Valine or Glycine or a conservative substitution thereof,

[00234] wherein the compound inhibits binding between iASPP and an iASPP binding partner selected from the group consisting of p53, p63 and p73.

[00235] In some embodiments, the peptides described herein are from about 8 amino acid residues in length to about 100 amino acid residues in length. Of course it is contemplated that longer or indeed shorter peptides also may prove useful. Thus, peptides of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 amino acids in length are contemplated. In some embodiments, a peptide described herein comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or more amino acids from iASPP added to its N-terminus. For example, the

amino acid at position 615 of SEQ ID NO: 8 could be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 1 amino acid to the N-terminus of a peptide sequence described herein is desired. Similarly, the amino acids at positions 611-615, 606-615, 601-615, or 596-615 of SEQ ID NO: 8 could be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 5, 10, 15, or 20 amino acids, respectively, to the N-terminus of the peptide is desired.

[00236] In some embodiments, a peptide described herein comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 or more amino acids added to its C-terminus. For example, the amino acid at position 624 of SEQ ID NO: 8 could be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 1 amino acid to the C-terminus of a peptide sequence described herein is desired. Similarly, the amino acids at positions 624-628, 624-633, 624-638 or 624-643 of SEQ ID NO: 8 could be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 5, 10, 15, or 20 amino acids, respectively, to the C-terminus of the peptide is desired.

[00237] In some embodiments, the addition of amino acids to both the N- and C-termini of a peptide described herein is contemplated. For example, the amino acids at positions 615 and 624 of SEQ ID NO: 8 can be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 1 amino acid to both the N- and C-termini of the peptide is desired. Similarly, the amino acids at positions 611-615 and 624-628; 606-615 and 624-633; 601-615 and 624-638; or 595-615 and 624-643 of SEQ ID NO: 8 can be added to a peptide described herein (e.g., SPRKARRA (SEQ ID NO: 2 or residues 616-623 of SEQ ID NO: 8), SPRKSRRA (SEQ ID NO: 3) or SPRRARRA (SEQ ID NO: 4)) if the addition of 5, 10, 15, or 20 amino acids, respectively, to the N- and C-termini of the peptide is desired.

[00238] In some embodiments, the peptide consists of an amino acid sequence at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least

90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to a peptide/polypeptide described herein.

[00239] In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 2, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRKARRA (SEQ ID NO: 2). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% , or more identical to SEQ ID NO: 3, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRKSRRRA (SEQ ID NO: 3). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 4, wherein the peptide retains the ability to interact with (bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRRARRA (SEQ ID NO: 4). In some embodiments, the compound is a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 5, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the compound is a peptide consisting of the amino acid sequence SPRRSRRRA (SEQ ID NO: 5).

[00240] Compounds of the invention include the peptide of SEQ ID NO: 2 and variants thereof. In some embodiments, variants of this peptide include those with one or more, e.g. 1, 2, 3, 4, 5 or more amino acid alterations. In some embodiments, the residues of which may be altered, and the alterations contemplated, may be selected from, for example, those set out in Table 2 above. For example, the alteration of position 5 of peptide P1 (iASPP residue 620 of SEQ ID NO: 8) to Ser (serine) provides the peptide of SEQ ID NO:3. The alteration of position 4 of the peptide P1 (iASPP residue 619 of SEQ ID NO: 8) to Arg (arginine) provides the peptide of SEQ ID NO:4. Alteration of both residues in the same peptide provides the peptide of SEQ ID NO:5. Other alterations, both individually and combinations thereof, may be made based on the alterations set out in Table 2 above.

[00241] In some embodiments, the variant peptides may differ in amino acid sequence by one or more substitutions, additions, deletions, truncations which may be present in any combination. In one aspect, the modification is a conservative substitution. The term

“conservative substitution” as used herein denotes the replacement of an amino acid residue by another, biologically similar residue with respect to hydrophobicity, hydrophilicity, cationic charge, anionic charge, shape, polarity and the like. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. The term “conservative substitution” also includes the use of a substituted or modified amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. By “substituted” or “modified” the present invention includes those amino acids that have been altered or modified from naturally occurring amino acids.

[00242] As such, it should be understood that in the context of the present invention, a conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in the Table A below:

Table 7
Conservative Substitutions I

<u>SIDE CHAIN</u> <u>CHARACTERISTIC</u>	<u>AMINO ACID</u>
Aliphatic	
Non-polar	G, A, P I, L, V
Polar - uncharged	C, S, T, M N, Q
Polar - charged	D, E K, R
Aromatic	H, F, W, Y
Other	N, Q, D, E

[00243] Alternatively, conservative amino acids can be grouped as described in Lehninger, [Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77] as set out in Table B, immediately below.

Table 8
Conservative Substitutions II

SIDE CHAIN	
<u>CHARACTERISTIC</u>	<u>AMINO ACID</u>
Non-polar (hydrophobic)	
A. Aliphatic:	A, L, I, V, P
B. Aromatic:	F, W
C. Sulfur-containing:	M
D. Borderline:	
G	
Uncharged-polar	
A. Hydroxyl:	S, T, Y
B. Amides:	N, Q
C. Sulfhydryl:	
C	
D. Borderline:	
G	
Positively Charged (Basic):	K, R, H
Negatively Charged (Acidic):	D, E

[00244] As still an another alternative, exemplary conservative substitutions are set out in Table 9, immediately below.

Table 9
Conservative Substitutions III

<u>Original Residue</u>	<u>Exemplary Substitution</u>
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn

Glu (E)	Asp
His (H)	Asn, Gln, Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe,
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser
Val (V)	Ile, Leu, Met, Phe, Ala

[00245] Any conservative substitution variant of SPRKARRA (SEQ ID NO: 2) is contemplated to be a useful peptide of the present invention as long as such a variant retains its property of being an inhibitor of binding of iASPP to an iASPP binding partner. Additionally it is contemplated that non-conservative substitution variants of these peptides also may be designed that may prove to be more efficient inhibitors of p53/p63/p73 binding to iASPP than the original SPRKARRA (SEQ ID NO: 2) described herein.

[00246] In some embodiments, the substituted amino acids are unnatural amino acids. For example, this includes D-amino acids of the amino acids of peptide P1 (SEQ ID NO: 2) or the alterations discussed above. Other unnatural amino acids include ornithine, diaminobutyric acid, ornithine, norleucine, ornithine, pyrrolysine, thienylalanine, naphthylalanine, phenylglycine, alpha and alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, halide derivatives of natural amino acids, such as trifluorotyrosine, p-Cl-phenylalanine, p-Br-phenylalanine, p-I-phenylalanine, L-allyl-glycine, beta-alanine, L-alpha-amino butyric acid, L-gamma-amino butyric acid, L-alpha-amino isobutyric acid, L-glutamic acid, 7-amino heptanoic acid, L-methionine sulfone, L-norleucine, L-norvaline, p-nitro-L-phenylalanine, L-hydroxyproline, L-thioprolin, methyl derivatives of phenylalanine – such as 1-methyl-Phe, pentamethyl-Phe, L-Phe(4-amino), L-

Tyr(methyl), L-Phe(4-isopropyl), L-Tic(1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid), L-diaminopropionic acid and L-Phe(4-benzyl).

[00247] Also contemplated are derivatives of SEQ ID NO: 2. Derivatives of such peptides form a further aspect of the invention. By “derivative” it is meant one of more of (a) addition of one or more polyalkyleneglycol (e.g. polyethylene glycol (PEG)) moieties to the peptide, e.g. to the N- or C-terminal of the peptide, (b) modification of an amino acid side-chain residue by, for example, esterification of an acid group (e.g. with a C₁-6 alkyl moiety) or the modification of an amine group to a mono- or di-C₁-6alkyl amine, (c) replacement of one or more amide bonds by ester or alkyl backbone bonds.

[00248] Peptides can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C₁-C₁₆ ester, or converted to an amide of formula NR₁R₂ wherein R₁ and R₂ are each independently H or C₁-C₁₆ alkyl, or combined to form a heterocyclic ring, such as a 5- or 6-membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C₁-C₁₆ alkyl or dialkyl amino or further converted to an amide. Peptides can also be modified by methylation. A peptide is considered methylated when methyl group is added at one or more nucleophilic side chains. Methylation in the proteins negates the negative charge on it and increase the hydrophobicity of the protein.

[00249] Hydroxyl groups of the peptide side chains may be converted to C₁-C₁₆ alkoxy or to a C₁-C₁₆ ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine or iodine, or with C₁-C₁₆ alkyl, C₁-C₁₆ alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C₂-C₄ alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this invention to select and provide conformational constraints to the structure that result in enhanced stability. For example, a carboxyl-terminal or amino-terminal cysteine residue can be added to the peptide, so

that when oxidized the peptide will contain a disulfide bond, generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters.

[00250] Peptidomimetic and organomimetic embodiments are also contemplated, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains, resulting in such peptido- and organomimetics of the proteins of this disclosure having measurable or enhanced ability to bind an antibody. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs", in Klegerman & Groves, eds., 1993, *Pharmaceutical Biotechnology*, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and *Principles of Pharmacology* Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in CADD. Also included within the scope of the disclosure are mimetics prepared using such techniques.

[00251] Peptides of the invention defined above may further be cyclized. Methods are well known in the art for introducing cyclic structures into the peptides of the present invention to select and provide conformational constraints to the structure that result in enhanced stability. For example, a C- or N-terminal cysteine can be added to the peptide, so that when oxidized the peptide will contain a disulfide bond, generating a cyclic peptide. Other peptide cyclizing methods include the formation of thioethers and carboxyl- and amino-terminal amides and esters. A number of synthetic techniques have been developed to generate synthetic circular peptides (Tam and Lu, *Protein Sci.*, 7(7) 1583-1592 (1998); Romanovskis and Spatola, *J. Pept. Res.*, 52(5) 356-374 (1998); Camarero and Muir, *J. Amer. Chem. Soc.*, 121 5597-5598 (1999); Valero, et al., *J. Pept. Res.*, 53(1) 56-67 (1999)). The role of cyclizing peptides is two-fold: a) to reduce hydrolysis in vivo and b) to thermodynamically destabilize the unfolded state and promote secondary structure formation.

[00252] Preferably, the peptides described herein are non-hydrolyzable. To provide such peptides, one may select peptides from a library non-hydrolyzable peptides, such as peptides containing one or more D-amino acids or peptides containing one or more non-hydrolyzable peptide bonds linking amino acids. Alternatively, one can select peptides which are optimal for

inhibiting iASPP binding to an iASPP binding partner and then modify such peptides as necessary to reduce the potential for hydrolysis by proteases. For example, to determine the susceptibility to proteolytic cleavage, peptides may be labeled and incubated with cell extracts or purified proteases and then isolated to determine which peptide bonds are susceptible to proteolysis, e.g., by sequencing peptides and proteolytic fragments. Alternatively, potentially susceptible peptide bonds can be identified by comparing the amino acid sequence of the inhibitory peptides of the present invention with the known cleavage site specificity of a panel of proteases. Based on the results of such assays, individual peptide bonds which are susceptible to proteolysis can be replaced with non-hydrolyzable peptide bonds by in vitro synthesis of the peptide.

[00253] Many non-hydrolyzable peptide bonds are known in the art, along with procedures for synthesis of peptides containing such bonds. Non-hydrolyzable bonds include --[CH₂NH]-- reduced amide peptide bonds, --[COCH₂]-- ketomethylene peptide bonds, --[CH(CN)NH]-- (cyanomethylene)amino peptide bonds, --[CH₂CH(OH)]-- hydroxyethylene peptide bonds, --[CH₂O]-- peptide bonds, and --[CH₂S]-- thiomethylene peptide bonds (see *e.g.*, U.S. Patent 6,172,043).

[00254] Furthermore, nonpeptide analogs of peptides which provide a stabilized structure or lessened biodegradation, are also contemplated. Peptide mimetic analogs can be prepared based on a selected inhibitory peptide by replacement of one or more residues by nonpeptide moieties. Preferably, the nonpeptide moieties permit the peptide to retain its natural confirmation, or stabilize a preferred, *e.g.*, bioactive, confirmation. One example of methods for preparation of nonpeptide mimetic analogs from peptides is described in Nachman *et al.*, *Regul. Pept.* 57:359-370 (1995). Peptide as used herein embraces all of the foregoing.

[00255] In particular, it is anticipated that the peptides described herein can be conjugated to a reporter group, including, but not limited to a radiolabel, a fluorescent label, an enzyme (*e.g.*, that catalyzes a colorimetric or fluorometric reaction), a substrate, a solid matrix, or a carrier (*e.g.*, biotin or avidin). The invention accordingly provides a molecule comprising a peptide inhibitor of iASPP binding to an iASPP binding partner, wherein the molecule preferably further comprises a reporter group selected from the group consisting of a radiolabel, a fluorescent label, an enzyme, a substrate, a solid matrix, and a carrier. Such labels are well known to those of skill in the art, *e.g.*, biotin labels are particularly contemplated. The use of such labels is well known to those of skill in the art and is described in, *e.g.*, U.S. No. Patent 3,817,837; U.S. Patent

No. 3,850,752; U.S. Patent No. 3,996,345 and U.S. Patent No. 4,277,437. Other labels that will be useful include but are not limited to radioactive labels, fluorescent labels and chemiluminescent labels. U.S. Patents concerning use of such labels include for example U.S. Patent No. 3,817,837; U.S. Patent No. 3,850,752; U.S. Patent No. 3,939,350 and U.S. Patent No. 3,996,345. Any of the peptides of the present invention may comprise one, two, or more of any of these labels.

[00256] The peptides described herein can be used as therapeutic compositions either alone or in combination with other therapeutic agents. For such therapeutic uses small molecules are generally preferred because the reduced size renders such peptides more accessible for uptake by the target.

[00257] Peptides of the invention may be prepared using methods known in the art. For example, peptides may be produced by chemical synthesis, eg. solid phase techniques and automated peptide synthesizers, or by recombinant means (using nucleic acids such as those described herein). For example, peptides may be synthesized using solid phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. The peptides can then be purified by reversed phase-HPLC and lyophilized. The peptide may be prepared by cleavage of a longer peptide. Thus, the peptide may be a fragment of the iASPP C-terminal sequence described herein.

[00258] Peptides may also be prepared by recombinant expression of the polynucleotides described herein. Peptides are expressed in suitable host cells and isolated using methods known in the art.

[00259] Fusion proteins comprising a peptide described herein, and a heterologous polypeptide, are specifically contemplated. Nonlimiting examples of heterologous polypeptides which can be fused to polypeptides of interest include proteins with long circulating half-life, such as, but not limited to, immunoglobulin constant regions (e.g., Fc region); transthyretin (WO 2003/086444, the disclosure of which is incorporated herein by reference in its entirety), marker sequences that permit identification of the polypeptide of interest; sequences that facilitate purification of the polypeptide of interest; and sequences that promote formation of multimeric proteins. In some embodiments, a receptor fragment is fused to alkaline phosphatase (AP). Methods for making Fc or AP fusion constructs are found in WO 02/060950, the disclosure of which is incorporated herein in its entirety.

[00260] Methods of making antibody fusion proteins are well known in the art. See, e.g., U.S. Patent No. 6,306,393, the disclosure of which is incorporated herein by reference in its entirety. In certain embodiments of the invention, fusion proteins are produced which may include a flexible linker, which connects the chimeric scFv antibody to the heterologous protein moiety. Appropriate linker sequences are those that do not affect the ability of the resulting fusion protein to be recognized and bind the epitope specifically bound by the V domain of the protein (see, e.g., WO 98/25965, the disclosure of which is incorporated herein by reference in its entirety).

[00261] For further discussions of fusion proteins see, for example, WO 95/04076, published 9 February 1995; US. Patent 5,629,172 issued 13 May 1997; WO 94/23040, published 13 October 1994; Flaschel et al., *Biotech Adv.*, 11:31-78 (1993); European patent application 207,044, published 30 December 1986; US Patent 5,322,930, issued 21 June 1994; European Patent 293,249, published 30 November 1988; US Patent 5,654,176, issued 5 August 1997; WO 95/16044, published 15 June 1995; WO 94/02502, published 3 February 1994; and WO 92/13955, published 20 August 1992, the disclosures of which are incorporated herein by reference in their entireties.

(vii) Nucleic acids, vectors and host cells

[00262] In a further aspect, the invention provides a nucleic acid molecule comprising a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at 81%, at least 82%, at least 83%, at least 84%, at least 85%, least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to a peptide/polypeptide described herein.

[00263] In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 2, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRKARRA (SEQ ID NO: 2). In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 3, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the

nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRKSRRRA (SEQ ID NO: 3). In some embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 4, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding a peptide consisting of the amino acid sequence SPRRARRA (SEQ ID NO: 4). In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of an amino acid sequence at least 50%, at least 62.5%, at least 75%, at least 87.5% ,or more identical to SEQ ID NO: 5, wherein the peptide retains the ability to interact with (e.g., bind to) iASPP. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes a peptide consisting of the amino acid sequence SPRRSRRRA (SEQ ID NO: 5).

[00264] In one embodiment, the nucleic acid is operably linked to a promoter. In a further embodiment, the nucleic acid, optionally operably linked to a promoter, has a stop codon immediately adjacent to the final codon of the sequence encoding SEQ ID NO: 2, 3, 4 or 5.

[00265] Complementary molecules are useful as templates for synthesizing coding molecules, and for making stable double-stranded polynucleotides. Due to the well-known degeneracy of the universal genetic code, one can synthesize numerous polynucleotide sequences that encode each polypeptide of the present invention. All such polynucleotides are contemplated as part of the invention. Such polynucleotides are useful for recombinant expression of polypeptides of the invention *in vivo* or *in vitro* (e.g., for gene therapy).

[00266] This genus of polynucleotides embraces polynucleotides comprises nucleotide sequences that encode peptides/polypeptides with one or a few amino acid differences (additions, insertions, or deletions) relative to amino acid sequences specifically depicted herein. Such changes are easily introduced by performing site directed mutagenesis, for example.

[00267] Polynucleotides of the invention (and peptides/polypeptides encoded thereby) can be defined by molecules that hybridize under specified conditions to a polynucleotide sequence complementary to a sequence that encodes a construct of the invention.

[00268] Exemplary highly stringent hybridization conditions are as follows: hybridization at 65°C for at least 12 hours in a hybridization solution comprising 5X SSPE, 5X Denhardt's, 0.5% SDS, and 2 mg sonicated non homologous DNA per 100 ml of hybridization solution;

washing twice for 10 minutes at room temperature in a wash solution comprising 2X SSPE and 0.1% SDS; followed by washing once for 15 minutes at 65°C with 2X SSPE and 0.1% SDS; followed by a final wash for 10 minutes at 65°C with 0.1X SSPE and 0.1% SDS. Moderate stringency washes can be achieved by washing with 0.5X SSPE instead of 0.1X SSPE in the final 10 minute wash at 65°C. Low stringency washes can be achieved by using 1X SSPE for the 15 minute wash at 65°C, and omitting the final 10 minute wash. It is understood in the art that conditions of equivalent stringency can be achieved through variation of temperature and buffer, or salt concentration as described Ausubel, et al. (Eds.), *Protocols in Molecular Biology*, John Wiley & Sons (1994), pp. 6.0.3 to 6.4.10. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and the percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook et al., (Eds.), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

[00269] Preferred variants exhibit the activities of native molecules described herein, such as the ability to inhibit the binding between iASPP and an iASPP binding partner (i.e., p53, p63 or p73).

[00270] In a related embodiment, the invention provides vectors comprising a polynucleotide of the invention. Such vectors are useful, e.g., for amplifying the polynucleotides in host cells to create useful quantities thereof, and for expressing polypeptides of the invention using recombinant techniques. There is a significant amount of published literature with respect to expression vector construction and recombinant DNA techniques in general. Please see, Sambrook et al., (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) *DNA Cloning Techniques: A Practical Approach* Vol EU IRL Press, Oxford UK; DNA Cloning: F M Ausubel et al, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (1994). In preferred embodiments, the vector is an expression vector wherein the polynucleotide of the invention is operatively linked to a polynucleotide comprising an expression control sequence. Autonomously replicating recombinant expression constructs such as plasmid and viral DNA vectors incorporating polynucleotides of the invention are specifically contemplated. Expression control DNA sequences include promoters, enhancers, and operators, and are generally selected based on the expression systems in which the expression construct is to be utilized. Preferred promoter and enhancer sequences are generally selected for the ability to increase gene expression, while operator sequences are generally selected for the ability to

regulate gene expression. Expression vectors are useful for recombinant production of polypeptides of the invention. Expression constructs of the invention may also include sequences encoding one or more selectable markers that permit identification of host cells bearing the construct. Expression constructs may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell. Preferred constructs of the invention also include sequences necessary for replication in a host cell.

[00271] In some embodiments, polynucleotides of the invention further comprise additional sequences to facilitate the gene therapy. In one embodiment, a “naked” transgene encoding a polypeptide of the invention (i.e., a transgene without a viral, liposomal, or other vector to facilitate transfection) is employed for gene therapy. In this embodiment, the polynucleotide of the invention preferably comprises a suitable promoter and/or enhancer sequence (e.g., cytomegalovirus promoter/enhancer [Lehner et al., *J. Clin. Microbiol.*, 29:2494-2502 (1991); Boshart et al., *Cell*, 41:521-530 (1985)]; Rous sarcoma virus promoter [Davis et al., *Hum. Gene Ther.*, 4:151 (1993)]; Tie promoter [Korhonen et al., *Blood*, 86(5): 1828-1835 (1995)]; or simian virus 40 promoter) for expression in the target mammalian cells, the promoter being operatively linked upstream (i.e., 5') of the polypeptide coding sequence. The polynucleotides of the invention also preferably further includes a suitable polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation sequence) operably linked downstream (i.e., 3') of the polypeptide coding sequence. The polynucleotides of the invention also preferably comprise a nucleotide sequence encoding a secretory signal peptide fused in frame with the polypeptide sequence. The secretory signal peptide directs secretion of the polypeptide of the invention by the cells that express the polynucleotide, and is cleaved by the cell from the secreted polypeptide. The signal peptide sequence can be that of another secreted protein, or can be a completely synthetic signal sequence effective to direct secretion in cells of the mammalian subject.

[00272] The polynucleotide may further optionally comprise sequences whose only intended function is to facilitate large scale production of the vector, e.g., in bacteria, such as a bacterial origin of replication and a sequence encoding a selectable marker. However, in a preferred embodiment, such extraneous sequences are at least partially cleaved off prior to administration to humans according to methods of the invention. One can manufacture and administer such polynucleotides for gene therapy using procedures that have been described in the literature for other transgenes. See, e.g., Isner et al., *Circulation*, 91: 2687-2692 (1995); and Isner et al., *Human Gene Therapy*, 7: 989-1011 (1996); incorporated herein by reference in their entirety.

[00273] Vectors also are useful for “gene therapy” treatment regimens, wherein a polynucleotide that encodes a polypeptide of the invention is introduced into a subject in need of treatment involving the inhibition of the binding of iASPP to an iASPP binding partner (i.e., p53, p63 or p73) in a form that causes cells in the subject to express the polypeptide of the invention *in vivo*. Gene therapy aspects that are described in WO 2007/006573, the disclosure of which is incorporated herein by reference, are also applicable herein.

[00274] Any suitable vector may be used to introduce a polynucleotide that encodes a polypeptide of the invention encoding one of the polypeptides of the invention, into the host. Exemplary vectors that have been described in the literature include replication deficient retroviral vectors, including but not limited to lentivirus vectors [Kim et al., J. Virol., 72(1): 811-816 (1998); Kingsman & Johnson, Scrip Magazine, October, 1998, pp. 43 46.]; adeno-associated viral (AAV) vectors [U.S. Patent No. 5,474,935; U.S. Patent No. 5,139,941; U.S. Patent No. 5,622,856; U.S. Patent No. 5,658,776; U.S. Patent No. 5,773,289; U.S. Patent No. 5,789,390; U.S. Patent No. 5,834,441; U.S. Patent No. 5,863,541; U.S. Patent No. 5,851,521; U.S. Patent No. 5,252,479; Gnatenko et al., J. Invest. Med., 45: 87 98 (1997)]; adenoviral (AV) vectors [See, e.g., U.S. Patent No. 5,792,453; U.S. Patent No. 5,824,544; U.S. Patent No. 5,707,618; U.S. Patent No. 5,693,509; U.S. Patent No. 5,670,488; U.S. Patent No. 5,585,362; Quantin et al., Proc. Natl. Acad. Sci. USA, 89: 2581 2584 (1992); Stratford Perricadet et al., J. Clin. Invest., 90: 626 630 (1992); and Rosenfeld et al., Cell, 68: 143 155 (1992)]; an adenoviral adenoassociated viral chimeric (see for example, U.S. Patent No. 5,856,152) or a vaccinia viral or a herpesviral (see for example, U.S. Patent No. 5,879,934; U.S. Patent No. 5,849,571; U.S. Patent No. 5,830,727; U.S. Patent No. 5,661,033; U.S. Patent No. 5,328,688; Lipofectin mediated gene transfer (BRL); liposomal vectors [See, e.g., U.S. Patent No. 5,631,237 (Liposomes comprising Sendai virus proteins)] ; and combinations thereof. All of the foregoing documents are incorporated herein by reference in their entirety. Replication deficient adenoviral vectors constitute a preferred embodiment.

[00275] In another related embodiment, the invention provides host cells, including prokaryotic and eukaryotic cells, that are transformed or transfected (stably or transiently) with polynucleotides of the invention or vectors of the invention. Polynucleotides of the invention may be introduced into the host cell as part of a circular plasmid, or as linear DNA comprising an isolated protein coding region or a viral vector. Methods for introducing DNA into the host cell, which are well known and routinely practiced in the art include transformation, transfection, electroporation, nuclear injection, or fusion with carriers such as liposomes,

micelles, ghost cells, and protoplasts. As stated above, such host cells are useful for amplifying the polynucleotides and also for expressing the polypeptides of the invention encoded by the polynucleotide. The host cell may be isolated and/or purified. The host cell also may be a cell transformed *in vivo* to cause transient or permanent expression of the polypeptide *in vivo*. The host cell may also be an isolated cell transformed *ex vivo* and introduced post-transformation, e.g., to produce the polypeptide *in vivo* for therapeutic purposes. The definition of host cell explicitly excludes a transgenic human being.

[00276] Such host cells are useful in assays as described herein. For expression of polypeptides of the invention, any host cell is acceptable, including but not limited to bacterial, yeast, plant, invertebrate (e.g., insect), vertebrate, and mammalian host cells. For developing therapeutic preparations, expression in mammalian cell lines, especially human cell lines, is preferred. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., glycosylation, truncation, lipidation, and phosphorylation) as may be desirable to confer optimal biological activity on recombinant expression products of the invention. Glycosylated and non-glycosylated forms of polypeptides are embraced by the present invention. Similarly, the invention further embraces polypeptides described above that have been covalently modified to include one or more water soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol.

[00277] In a further embodiment, the nucleic acid encodes a cleavable leader sequence in-between the promoter and the sequence encoding SEQ ID NO:2, 3, 4 or 5 such that on expression in a host cell, the leader sequence is cleaved in order to provide a peptide consisting of any one of SEQ ID NO:2, 3, 4, and 5. The leader sequence may be heterologous to the peptide sequence. A number of leader sequences of such types are known in the art and may be selected to be compatible with the host cell in which the nucleic acid is expressed.

[00278] Alternatively, as noted above, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus tripartite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

[00279] Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) Cell 41:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In Transcription and splicing (ed. B. D. Hames and D. M. Glover); Proudfoot (1989) Trends Biochem. Scip. 14:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In Molecular Cloning: A Laboratory Manual].

[00280] Gene therapy vehicles for delivery of constructs including a nucleic acid of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picomavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) Cancer Gene Therapy 1:51-64; Kimura (1994) Human Gene Therapy 5:845-852; Connelly (1995) Human Gene Therapy 6:185-193; and Kaplitt (1994) Nature Genetics 6:148-153.

[00281] Thus, in one embodiment, the invention provides a method of treatment of a subject, which method comprises administration of a gene therapy vector encoding a peptide of any one of SEQ ID NO :2, 3, 4 and 5 or any other peptide described herein. The gene therapy vector may incorporate a nucleic acid of the invention in any of the forms described above. The treatment may be the treatment of a cancer, as described above. The treatment may be preceded by a diagnostic step of determining a sample of a tumor present in the patient, whether or not at least a portion of the tumor cells have wild-type p53. Optionally the status (i.e. wild-type or mutant) of p63 and/or p73 may be determined.

(viii) Antibodies

[00282] In another embodiment, the isolated compound is an antibody. Thus, the invention contemplates use of antibodies (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, humanized antibodies, human antibodies, and complementarity determining region (CDR)-grafted antibodies, including compounds that include CDR sequences specifically recognizing a polypeptide of the invention). Antibodies can be human antibodies which are produced and identified according to methods described in WO 93/11236, which is incorporated herein by reference in its entirety. Antibody fragments, including Fab, Fab', F(ab')₂, and Fv, and single-chain antibodies are also provided by the invention. The terms "specific for" or "specifically binds," when used to describe antibodies of the invention, indicates that the variable regions of the antibodies of the invention recognize and bind the polypeptide of interest with a detectable preference (i.e., able to distinguish the polypeptide of interest from other known polypeptides of the same family, by virtue of measurable differences in binding affinity, despite the possible existence of localized sequence identity, homology, or similarity between family members). It will be understood that specific antibodies may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays to determine binding specificity of an antibody of the invention are well known and routinely practiced in the art. For a comprehensive discussion of such assays, see Harlow et al. (Eds), *Antibodies A Laboratory Manual*; Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988), Chapter 6. Antibodies of the invention can be produced using any method well known in the art.

[00283] Various procedures known in the art may be used for the production of polyclonal antibodies to peptides described herein. For the production of antibodies, various host animals (including but not limited to rabbits, mice, rats, hamsters, and the like) are immunized by injection with a phosphorylated iASPP protein or peptide. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete) adjuvant, mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*Bacillus Calmette-Guerin*) and *Corynebacterium parvum*.

[00284] A monoclonal antibody to a peptide described herein (e.g., SEQ ID NOs: 2-5) may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Köhler *et al.*, *Nature*, 256: 495-497 (1975), and the more recent human B-cell hybridoma technique [Kosbor *et al.*, *Immunology Today*, 4: 72 (1983)] and the EBV-hybridoma technique [Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R Liss, Inc., pp. 77-96 (1985), all specifically incorporated herein by reference]. Antibodies against a peptide described herein also may be produced in bacteria from cloned immunoglobulin cDNAs. With the use of the recombinant phage antibody system it may be possible to quickly produce and select antibodies in bacterial cultures and to genetically manipulate their structure.

[00285] When the hybridoma technique is employed, myeloma cell lines may be used. Such cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and exhibit enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). For example, where the immunized animal is a mouse, one may use P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with cell fusions. It should be noted that the hybridomas and cell lines produced by such techniques for producing the monoclonal antibodies are contemplated compositions of the present invention.

[00286] In addition to the production of monoclonal antibodies, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used [Morrison *et al.*, *Proc. Natl. Acad. Sci.* 81:6851-6855 (1984); Neuberger *et al.*, *Nature* 312:604-608 (1984); Takeda *et al.*, *Nature* 314:452-454 (1985)]. Alternatively, techniques described for the production of single-chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce phosphorylated-iASPP peptide-specific single chain antibodies.

[00287] Antibody fragments which contain the idiotype of the molecule may be generated by known techniques. For example, such fragments include, but are not limited to, the F(ab')₂ fragment which may be produced by pepsin digestion of the antibody molecule; the Fab' fragments which may be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and

the two Fab fragments which may be generated by treating the antibody molecule with papain and a reducing agent.

[00288] Non-human antibodies may be humanized by any methods known in the art. A preferred "humanized antibody" has a human constant region, while the variable region, or at least a CDR, of the antibody is derived from a non-human species. Methods for humanizing non-human antibodies are well known in the art. (*see* U.S. Patent Nos. 5,585,089, and 5,693,762). Generally, a humanized antibody has one or more amino acid residues introduced into its framework region from a source which is non-human. Humanization can be performed, for example, using methods described in Jones *et al.*, *Nature* 321: 522-525, (1986), Riechmann *et al.*, *Nature*, 332: 323-327, (1988) and Verhoeven *et al.*, *Science* 239:1534-1536, (1988), by substituting at least a portion of a rodent complementarity-determining region for the corresponding regions of a human antibody. Numerous techniques for preparing engineered antibodies are described, *e.g.*, in Owens *et al.*, *J. Immunol. Meth.*, 168:149-165, (1994). Further changes can then be introduced into the antibody framework to modulate affinity or immunogenicity.

[00289] Rapid, large-scale recombinant methods for generating antibodies may be employed, such as phage display [Hoogenboom *et al.*, *J. Mol. Biol.* 227: 381, (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581, (1991)] or ribosome display methods, optionally followed by affinity maturation [see, *e.g.*, Ouwehand *et al.*, *Vox Sang* 74(Suppl 2):223-232 (1998); Rader *et al.*, *Proc. Natl. Acad. Sci. USA* 95:8910-8915 (1998); Dall'Acqua *et al.*, *Curr. Opin. Struct. Biol.* 8:443-450, (1998)]. Phage-display processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in WO 99/10494, which describes the isolation of high affinity and functional agonistic antibodies for MPL and msk receptors using such an approach.

[00290] Recombinant antibody fragments, *e.g.*, scFvs, can also be engineered to assemble into stable multimeric oligomers of high binding avidity and specificity to different target antigens. Such diabodies (dimers), triabodies (trimers) or tetrabodies (tetramers) are well known in the art, see *e.g.*, Kortt *et al.*, *Biomol Eng.* 2001 18:95-108, (2001) and Todorovska *et al.*, *J Immunol Methods.* 248:47-66, (2001).

Further therapeutically active moieties

[00291] In a further embodiment of the present invention the compound is linked to a therapeutically active moiety, preferably the moiety is cytotoxic.

[00292] The term "therapeutically active moiety" encompasses a moiety having beneficial, prophylactic and/or therapeutic properties. Methods of conjugating polypeptides to therapeutic agents are well known in the art. In one embodiment the therapeutically active moiety is a cytotoxic chemotherapeutic agent.

[00293] Cytotoxic chemotherapeutic agents are well known in the art. Exemplary cytotoxic chemotherapeutic agents, such as anticancer agents, include: alkylating agents including nitrogen mustards such as mechlorethamine (HN2), cyclophosphamide, ifosfamide, melphalan (L- sarcolysin) and chlorambucil; 10 ethylenimines and methylmelamines such as hexamethylmelamine, thiotepa; alkyl sulphonates such as busulfan; nitrosoureas such as carmustine (BCNU), lomustine (CCNLJ), semustine (methyl-CCN-U) and streptozoein (streptozotocin); and triazenes such as decarbazine (DTIC; dimethyltriazenoimidazolecarboxamide); Antimetabolites including folic acid analogues such as methotrexate (amethopterin); pyrimidine analogues such as fluorouracil (5- fluorouracil; 5-FU), floxuridine (fluorodeoxyuridine; FUdR) and cytarabine (cytosine arabinoside); and purine analogues and related inhibitors such as mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG) and pentostatin (2'-deoxycofonnycin). Natural Products including vinca alkaloids such as vinblastine (VLB) and vincristine; epipodophyllotoxins such as etoposide and teniposide; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin) and mitomycin (mitomycin Q; enzymes such as L-asparaginase; and biological response modifiers such as interferon alphenomes. Miscellaneous agents including platinum coordination complexes such as cisplatin (cis-DDP) and carboplatin; anthracenedione such as mitoxantrone and antbracycline; substituted urea such as hydroxyurea; methyl hydrazine derivative such as procarbazine (N-methylhydrazine, MIH); and adrenocortical suppressant such as mitotane (o, p'-DDD) and aminogluthethimide; taxol and analogues/derivatives; and hormone agonists/antagonists such as flutamide and tamoxifen.

[00294] In a further embodiment of the invention, the cytotoxic moiety is a cytotoxic peptide or polypeptide moiety by which we include any moiety which leads to cell death.

[00295] Cytotoxic peptide and polypeptide moieties are well known in the art and include, for example, ricin, abrin, *Pseudomonas* exotoxin, tissue factor and the like. The use of ricin as a cytotoxic agent is described in Burrows & Thorpe (1993) Proc. Natl. Acad. Sci. USA 90, 8996-9000, incorporated herein by reference, and the use of tissue factor, which leads to localised blood clotting and infarction of a tumour, has been described by Ran et al (1998) Cancer Res. 58, 4646-4653 and Huang et al (1997) Science 275, 25 547-550. Tsai et al (1995) Dis. Colon Rectum 38, 1067- 1074 describes the abrin A chain conjugated to a monoclonal antibody and is incorporated herein by reference. Other ribosome inactivating proteins are described as cytotoxic agents in WO 96/06641. *Pseudomonas* exotoxin may also be used as the cytotoxic polypeptide moiety (see, for example, Aiello et al (1995) Proc. Natl. Acad. Sci. USA 92, 10457-10461).

[00296] Certain cytokines, such as TNF α and IL-2, may also be useful as cytotoxic agents.

[00297] Certain radioactive atoms may also be cytotoxic if delivered in sufficient doses. Thus, the cytotoxic moiety may comprise a radioactive atom which, in use, delivers a sufficient quantity of radioactivity to the target site so as to be cytotoxic. Suitable radioactive atoms include phosphorus-32, iodine-125, iodine-131, indium-111, rhenium-186, rhenium-188 or yttrium-90, or any other isotope which emits enough energy to destroy neighboring cells, organelles or nucleic acid. Preferably, the isotopes and density of radioactive atoms in the compound of the invention are such that a dose of more than 4000 cGy (preferably at least 6000, 8000 or 10000 cGy) is delivered to the target site and, preferably, to the cells at the target site and their organelles, particularly the nucleus. The radioactive atom may be attached to the binding moiety in known ways. For example EDTA or another chelating agent may be attached to the binding moiety and used to attach ^{111}In or ^{90}Y . Tyrosine residues may be labelled with ^{125}I or ^{131}I .

[00298] Alternatively, any of these systems can be incorporated into a prodrug system. Such prodrug systems are well known in the art.

(v) *Screening methods for modulators of binding of iASPP to an iASPP binding partner*

[00299] Methods are provided for screening for modulators of binding of an iASPP polypeptide comprising a structure defined by the structural coordinates provided in Table 1 with an iASPP binding partner. Generally, such methods involve introducing one or more test compounds (alone or simultaneously) into a controlled system containing an iASPP polypeptide

and one of its binding partners, to determine whether the test compound affects binding between the iASPP and its natural binding partner. The assays can be quantitative in an absolute sense, involving measurements of binding, or quantitative in a relative sense, involving comparative measurements of binding, such as comparative evaluation of the intensity of a radioactive or colorimetric signal. It is often beneficial, but not absolutely necessary, to compare the results involving a test compound with a control involving no test compound to evaluate the effects of the compound. However, even absent such a control, the binding measurement can provide an indication of the effect of the test compound, and the effect of the test compound can alternatively be compared to results involving previous test compounds or known binding inhibitors.

[00300] Such assays can be performed *in vitro* in cell-free formats or cell based formats, which often lend themselves to high throughput implementation. In some cell-free variations, the iASPP or the binding partner is attached to a solid support (e.g., a bead, membrane, plate, or chip) and the other, unattached moiety is incubated with the solid support to permit binding. Optionally, the unbound moiety (the iASPP or the binding partner) is labeled (radiolabel, colorimetric label, etc.) or tagged (e.g., a peptide or epitope tag, or GFP fusion or enzymatic fusion) to facilitate measurement of binding.

[00301] Cell-based binding assays can involve cells that naturally express one or both of the proteins (iASPP and its binding partner), or cells in which expression is induced, or cells which express one or both proteins due to recombinant modification, e.g., the introduction of an expression vector. Prokaryotic (e.g., bacteria such as *E. coli*) and Eukaryotic (e.g., animal, plant, yeast/fungal, etc.) cells can be employed. The test compound is introduced into the cell growth media or directly into the cell to determine its effect on binding.

[00302] In one aspect, the method comprises (a) contacting an iASPP polypeptide comprising a structure defined by the structural coordinates provided in Table 1 with an iASPP binding partner, in the presence and absence of a test compound, under conditions in which the binding partner binds with the iASPP polypeptide; and (b) comparing binding of the polypeptide and the binding partner in the presence and absence of the test compound. In another aspect, the method comprises measuring binding between a polypeptide that comprises comprising a structure defined by the structural coordinates provided in Table 1 with an iASPP binding partner with an iASPP binding partner, in the presence and absence of a test compound, under conditions in which the binding partner binds with the iASPP polypeptide. Increased binding of the

polypeptide to the binding partner identifies the test compound as an agonist of binding and decreased binding of the polypeptide to the binding partner identifies the test compound of as an antagonist of binding. In one aspect, the binding partner comprises a member selected from the group consisting of p53, p63 and p73.

[00303] In the case of binding partners identified herein, experiments can be performed with binding partners from any organism in which iASPP and the binding partner are expressed. Mammalian iASPP and binding partners are preferred, with primate highly preferred and human very highly preferred. Numerous sequences have already been reported in the scientific literature, public (Genbank, patent), and commercial databases.

[00304] In certain aspects, the binding partner comprises an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 98%, at least 99% or more identical an amino acid sequence of a mammalian protein selected from the group consisting of p53 (Genbank Accession Nos. BAC16799 (human), BAA82344 (mouse), BAA788379 (canine)), p63 (Genbank Accession Nos. AAB21139 (human) and Q9JJP6 (rat) and p73 (Genbank Accession Nos.: CAA72221 (human) and AAD33213 (mouse)).

[00305] In some variations, a full-length iASPP polypeptide (i.e., amino acids 1-828 of SEQ ID NO: 7) and the complete binding partner is employed in the assay. However, evidence, including that described below, indicates that the iASPP binding partners identified herein interact with a carboxyl-terminal portion of iASPP, and thus it is unnecessary to use the complete iASPP for binding assays of the invention. In the context of assays of the invention, the term "iASPP carboxyl-terminal domain" refers to a portion of iASPP that is sufficient to exhibit the activity (e.g., binding activity) needed for the assay. It is possible to determine a minimum effective iASPP carboxyl-terminal domain by screening deletion fragments of iASPP according to binding assay procedures described herein. The iASPP carboxyl-terminal domain can be fused to other sequences to make fusion proteins, and/or contain additional moieties such as labels and tags.

[00306] In one aspect, the polypeptide comprising an iASPP carboxyl-terminal domain comprises amino acids 1-828 of SEQ ID NO: 7 or comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at

least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to amino acids 1-828 of SEQ ID NO: 7.

[00307] In another aspect, the polypeptide comprising an iASPP carboxyl-terminal domain comprises amino acids 608-828 of SEQ ID NO: 7 or comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to amino acids 608-828 of SEQ ID NO: 7.

[00308] In other aspects, the polypeptide comprising an iASPP carboxyl-terminal domain comprises amino acids 616-623 of SEQ ID NO: 7 or comprises an amino acid sequence at least at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more identical to amino acids 616-623 of SEQ ID NO: 7.

[00309] Exemplary assays for screening test compounds include, but are not limited to, labeled *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays, and cell-based assays such as two- or three-hybrid screens and those used in high-throughput screening and expression assays. For example, hybrid screens can be used to rapidly examine the effect of transfected nucleic acids on the intracellular binding of iASPP polypeptides or fragments thereof to specific intracellular targets. The transfected nucleic acids can encode, for example, combinatorial peptide libraries or antisense molecules. Convenient reagents for such assays, such as GAL4 fusion proteins, are known in the art.

[00310] The polypeptides comprising an iASPP carboxyl-terminal domain used in the screening methods, when not produced by a transfected nucleic acid molecule in a cell based assay, can be added to an assay mixture as an isolated polypeptide. A polypeptide comprising an iASPP carboxyl-terminal domain can be produced recombinantly or isolated from biological extracts. Full-length or functional fragments of a polypeptide comprising an iASPP carboxyl-terminal domain can be used, as can mimetics and analogs thereof, as long as the portion, mimetic or analog provides binding affinity and avidity measurable in the assay.

[00311] The assay mixture also includes a test compound. In particular examples, a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a different response to the various concentrations. Typically, one of these concentrations serves as a

negative control (such as at zero concentration of agent or at a concentration of agent below the limits of assay detection). Exemplary test compounds are discussed below.

[00312] Additional reagents can be included in the mixture. Reagents such as salts, buffers, neutral proteins (such as albumin), and detergents, can be used to facilitate optimal protein-protein and/or protein-nucleic acid binding. Such a reagent can also reduce non-specific or background interactions of the reaction components. Other reagents that improve the efficiency of the assay such as protease, inhibitors, nuclease inhibitors, antimicrobial agents, and the like can also be used. Exemplary conditions include conditions that approximate reaction conditions inside a cell, and thus may include an isotonic level of salts, buffered pH, and avoidance of extreme temperatures.

[00313] The mixture of assay materials is incubated under conditions whereby, but for the presence of the test compound, the polypeptide comprising an iASPP carboxyl-terminal domain specifically binds the cellular binding target. Incubation temperatures typically are between 4°C and 40°C. Incubation times can be minimized to facilitate rapid, high throughput screening and such as about 0.1 to 10 hours.

[00314] After incubation, the presence or absence of specific binding between the polypeptide comprising an iASPP carboxyl-terminal domain and one or more binding partners is detected by any convenient method available to the user. Exemplary binding targets include, but are not limited to, p53, p63 and p73. For example, in a cell free binding assays, a separation step can be used to separate bound from unbound components. The separation step can be accomplished in a variety of ways. For example, at least one of the components can be immobilized on a solid substrate, from which the unbound components may be easily separated. The solid substrate can be made of a wide variety of materials and in a wide variety of shapes, such as a microtiter plate, a microbead, a dipstick, or a resin particle. Ideally, the substrate provides maximum signal to noise ratios, to minimize background binding.

[00315] In one example, separation is achieved by removing a bead or dipstick from a reservoir, emptying or diluting a reservoir such as a microtiter plate well, rinsing a bead, particle, chromatographic column or filter with a wash solution or solvent. The separation step can include multiple rinses or washes. For example, when the solid substrate is a microtiter plate, the wells can be washed several times with a washing solution, which typically includes those components of the incubation mixture that do not participate in specific bindings such as

salts, buffer, detergent, non-specific protein. Where the solid substrate is a magnetic bead, the beads can be washed one or more times with a washing solution and isolated using a magnet.

[00316] Detection of the presence or absence of a polypeptide comprising an iASPP carboxyl-terminal domain complex with a binding partner can be achieved using any method known in the art. For example, the transcript resulting from a reporter gene transcription assay of a polypeptide comprising an iASPP carboxyl-terminal domain interacting with a target molecule (e.g., binding partner) typically encodes a directly or indirectly detectable product (such as β -galactosidase activity, luciferase activity, and the like). For cell-free binding assays, one of the components usually includes, or is coupled to, a detectable label. A wide variety of labels can be used, such as those that provide direct detection (such as radioactivity, luminescence, optical or electron density) or indirect detection (such as epitope tag such as the FLAG epitope, enzyme tag such as horseradish peroxidase). The label can be bound to an iASPP binding partner, or incorporated into the structure of the binding partner.

[00317] A variety of methods can be used to detect the label, depending on the nature of the label and other assay components. For example, the label can be detected while bound to the solid substrate or subsequent to separation from the solid substrate. Labels can be directly detected through optical or electron density, radioactive emissions, nonradiative energy transfers or indirectly detected with antibody conjugates, or streptavidin-biotin conjugates. Methods for detecting the labels are well known in the art.

[00318] A test compound employed in a method of the invention can be any organic or inorganic chemical or biological molecule known in the art, such as small organic or inorganic molecules preferably found in small molecule libraries containing compounds of synthetic or natural origin, or combinatorial libraries as described below. Further, peptides, preferably found in peptide libraries, are contemplated as candidate modulators such as inhibitors. Test compounds suitable for administration as therapeutics will exhibit acceptable toxicity levels as would be known in the art or determinable by one of skill in the art using routine experimentation. Toxicity can be determined in subsequent assays, however, and often “designed out” of molecules by pharmaceutical chemists. Screening of chemical libraries such as those developed and maintained by pharmaceutical companies, consisting of both chemically synthesized and natural compounds, and combinatorial libraries, are specifically contemplated.

[00319] Chemical libraries may contain known compounds, proprietary structural analogs of known compounds, or compounds that are identified from natural product screening.

[00320] Natural product libraries are collections of materials isolated from natural sources, typically, microorganisms, animals, plants, or marine organisms. Natural products are isolated from their sources by fermentation of microorganisms followed by isolation and extraction of the fermentation broths or by direct extraction from the microorganism or tissue (plant or animal) themselves. Natural product libraries include polyketides, non-ribosomal peptides, and variants (including non-naturally occurring variants) thereof. See Cane et al., *Science*, 282:63-68 (1998), incorporated herein by reference.

[00321] Combinatorial libraries are composed of large numbers of related compounds, such as peptides, oligonucleotides, or other organic compounds as a mixture. Such compounds are relatively straightforward to design and prepare by traditional automated synthesis protocols, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries.

[00322] Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created thereby, see Myers, *Curr. Opin. Biotechnol.*, 8:701-707 (1997), incorporated herein by reference.

[00323] Modulators of iASPP binding to an iASPP binding partner identified by assessment of the test compounds may be formulated into compositions which include pharmaceutically acceptable (*i.e.*, sterile and non-toxic) liquid, semisolid, or solid diluents that serve as pharmaceutical vehicles, excipients, or media.

[00324] Compositions comprising one or more modulators of iASPP binding to an iASPP binding partner can be packaged in forms convenient for delivery. The compositions can be enclosed within a capsule, caplet, sachet, cachet, gelatin, paper, or other container. The dosage units can be packaged, *e.g.*, in tablets, capsules, suppositories or cachets.

Therapeutic uses of the Compounds

[00325] Following identification of a compound by the present invention, including a peptide of the invention referred to above, it may be manufactured and/or used in the preparation, *i.e.* manufacture or formulation, of a composition such as a medicament, pharmaceutical

composition or drug. Small peptides may be manufactured by recombinant DNA technology, as described further herein, or synthetically. These may be administered to individuals.

[00326] Thus, the present invention extends in various aspects not only to a compound as provided by the invention, but also a pharmaceutical composition, medicament, drug or other composition comprising such a compound for the treatment of a neoplastic disorder. Thus, in one aspect, a method of treating a neoplastic disorder comprising administering to a subject in need of treatment a composition comprising a compound of the invention in an amount effective to treat the neoplastic disorder is contemplated. In some embodiments, prior to the administering step, a subject having a neoplastic disorder is identified. The identifying step may comprise identifying a subject with a neoplastic disorder characterized by elevated expression of an iASPP polypeptide in neoplastic cells. In some embodiments, the identifying step comprises screening a biological sample suspected of containing neoplastic cells from the subject for elevated expression of the iASPP polypeptide. The screening step comprises comparing expression of iASPP in cells from the sample with expression of iASPP from healthy cells of the same type of cells in the sample, wherein increased expression of the iASPP in cells from the sample compared to the expression of the iASPP in cells from the healthy cells indicates elevated expression of iASPP.

[00327] In some embodiments, the neoplastic disorder is a cancer selected from the group consisting of breast cancer, pancreatic cancer, ovarian cancer, colorectal cancer, esophageal cancer, lung cancer, head cancer, neck cancer, gastric cancer and epithelial cancer,

[00328] In another embodiment, the invention provides a method of selecting a therapeutic regimen for a subject comprising identifying a subject as having a neoplastic disorder characterized by increased expression of an iASPP polypeptide and administering to the subject a compound that inhibits binding between iASPP and an iASPP binding partner. In some embodiments, the identifying step comprises screening a biological sample suspected of containing neoplastic cells from the subject for elevated expression of the iASPP polypeptide. The screening step comprises comparing expression of iASPP in cells from the sample with expression of iASPP from healthy cells of the same type of cells in the sample, wherein increased expression of the iASPP in cells from the sample compared to the expression of the iASPP in cells from the healthy cells indicates elevated expression of iASPP.

[00329] Also provided is a method of stimulating p53-mediated apoptosis in a cell comprising contacting the cell with a compound that inhibits binding between iASPP and an iASPP binding partner in an amount effective to stimulate p53-mediated apoptosis in the cell. In one embodiment, the cell expresses wild-type p53.

[00330] The compositions may be used for treatment (which may include preventative treatment) of disease such as cancer. Cancers include lung cancer, small cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, stomach cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, thyroid cancer, breast cancer, ovarian cancer, endometrial cancer, prostate cancer, testicular cancer, liver cancer, kidney cancer, renal cell carcinoma, bladder cancer, pancreatic cancer, brain cancer, glioma, sarcoma, osteosarcoma, bone cancer, skin cancer, squamous cancer, Kaposi's sarcoma, melanoma, malignant melanoma, lymphoma, and leukemia.

[00331] In one embodiment, the treatment is treatment of:

[00332] a carcinoma, for example a carcinoma of the bladder, breast, colon (e.g., colorectal carcinomas such as colon adenocarcinoma and colon adenoma), kidney, epidermal, liver, lung (e.g., adenocarcinoma, small cell lung cancer and non-small cell lung carcinomas), oesophagus, gall bladder, ovary, pancreas (e.g., exocrine pancreatic carcinoma), stomach, cervix, thyroid, prostate, skin (e.g., squamous cell carcinoma);

[00333] a hematopoietic tumour of lymphoid lineage, for example leukemia, acute lymphocytic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma, or Burkett's lymphoma;

[00334] a hematopoietic tumor of myeloid lineage, for example acute and chronic myelogenous leukemias, myelodysplastic syndrome, or promyelocytic leukemia;

[00335] a tumour of mesenchymal origin, for example fibrosarcoma or habdomyosarcoma;

[00336] a tumor of the central or peripheral nervous system, for example astrocytoma, neuroblastoma, glioma or schwannoma;

[00337] melanoma; seminoma; teratocarcinoma; osteosarcoma; xenoderoma pigmentoum; keratocanthoma; thyroid follicular cancer; or Kaposi's sarcoma.

[00338] In one embodiment, the treatment is treatment of solid tumour cancer (e.g., cancer characterized by the appearance of solid tumours).

[00339] The term “treatment,” as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g., in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, alleviation of symptoms of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis) is also included. For example, use with patients who have not yet developed the condition, but who are at risk of developing the condition, is encompassed by the term “treatment.”

[00340] For example, treatment of cancer includes the prophylaxis of cancer, reducing the incidence of cancer, alleviating the symptoms of cancer, etc.

[00341] Such a treatment may comprise administration of such a composition to a patient, e.g. for treatment of disease; the use of such an inhibitor in the manufacture of a composition for administration, e.g. for treatment of disease; and a method of making a pharmaceutical composition comprising admixing such a compound with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients. The administration of the agent will be of a therapeutically-effective amount, i.e. that amount of an active compound, or a material, composition or dosage form comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen.

Combination Therapy

[00342] In some embodiments, the methods of the invention further comprise the administration of a standard of care anti-neoplastic therapy selected from the group consisting of a standard of care therapeutic, a standard of care radiotherapeutic, or a standard of care radiation regimen for the neoplastic disorder. Thus, in one aspect, methods of treatment of a condition associated with tumor cell growth in a subject are provided comprising administering to the subject a compound that inhibits binding between iASPP and an iASPP binding partner and a standard of care anti-neoplastic therapy.

[00343] Administration of a combination of a compound that inhibits binding between iASPP and an iASPP binding partner with one or more additional therapeutics/second agents in methods of the invention may reduce the amount of either agent needed as a therapeutically effective dosage, and thereby reduce any negative side effects the agents may induce *in vivo*. Thus, a method of treating a neoplastic disorder comprising administering to a subject in need of treatment a composition comprising a compound of the invention in an amount effective to treat the neoplastic disorder and an additional therapeutic agent is specifically contemplated. Additional therapeutics or second agents contemplated for use in combination with an iASPP antagonist or an iASPP inhibitor peptide include a tyrosine kinase inhibitor, a cytokine, a chemotherapeutic agent, a radiotherapeutic agent, or radiation therapy.

[00344] Any tyrosine kinase inhibitor may be suitable for use in combination with an iASPP antagonist or an iASPP inhibitor peptide in a composition or method of the invention. Inhibitors of non-receptor tyrosine kinases are specifically contemplated. Examples of suitable tyrosine kinase inhibitors include, but are not limited to, SKI-606 (4-anilino-3-quinolinecarbonitrile), PD173955 (pyrido[2,3-d]pyrimidine), AZD0530, AZM475271, PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine), PP1 (4-Amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine), CGP76030 (substituted 5,7-diphenyl-pyrrolo[2,3d]pyrimidine), dasatinib, TG100435 ([7-(2,6-dichloro-phenyl)-5-methyl-benzo[1,2,4]triazin-3-yl]-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-amine), TG100855 [7-(2,6-dichloro-phenyl)-5-methyl-benzo[1,2,4]triazin-3-yl]-{4-[2-(1-oxy-pyrrolidin-1-yl)-ethoxy]-phenyl}-amine), AZM 475271, M475271, SU6656 (2,3-Dihydro-N,N-dimethyl-2-oxo-3-[(4,5,6,7-tetrahydro-1H-indol-2-yl)methylene]-1H-indole-5-sulfonamide), staurosporine from *Streptomyces staurosporeus*, adaphostin (4-Amino-N-(2,5-dihydroxybenzyl)adamantyl Benzoate, 4-(2,5-Dihydroxy-benzylamino)benzoic Acid Adamantan-1-yl Ester), Tyrphostin AG 957 (4-Amino-N-(2,5-dihydroxybenzyl)methyl benzoate), Imatinib mesylate (Gleevec®, Glivec®), aminogenistein (4'-Amino-6-Hydroxyflavone), Indirubin-3'-oxime (3-[1,3-Dihydro-3-(hydroxyimino)-2H-indol-2-ylidene]-1,3-dihydro-2H-indol-2-one), Kenpaullone (9-Bromo-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one), LFM-A13 (2-Cyano-N-(2,5-dibromophenyl)-3-hydroxy-2-butenamide), Bisindolylmaleimide IX, (Methanesulfonate Salt), edelfosine (rac-2-methyl-1-octadecyl-glycero-(3)-phosphocholine), Rapamycin, Quercetin (3,3',4',5,7-Pentahydroxyflavone . 2H₂O and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyrano-4-one), TBB (4,5,6,7-Tetrabromotriazole), and Triciribine (6-Amino-4-methyl-8-(β-D-ribofuranosyl)4H,8H-pyrrolo[4,3,2-de]pyrimido[4,5-c] pyridazine).

[00345] Any chemotherapeutic or radiotherapeutic agent may be suitable for use in combination with an iASPP antagonist or an iASPP inhibitor peptide in a composition or method of the invention, and may be identified by means well known in the art. Examples of suitable chemotherapeutic and radiotherapeutic agents include, but are not limited to: an anti-metabolite; a DNA-damaging agent; a cytokine useful as a chemotherapeutic agent; a covalent DNA-binding drug; a topoisomerase inhibitor; an anti-mitotic agent; an anti-tumor antibiotic; a differentiation agent; an alkylating agent; a methylating agent; a hormone or hormone antagonist; a nitrogen mustard; a radiosensitizer; a photosensitizer; a radiation source, optionally together with a radiosensitizer or photosensitizer; or other commonly used therapeutic agents.

[00346] Specific examples of chemotherapeutic agents useful in methods of the present invention are listed in Table 10 below.

TABLE 10		
<u>Alkylating agents</u>	<u>Epipodophylotoxins</u>	<u>Hormones and antagonists</u>
<u>Nitrogen mustards</u>	etoposide	<u>Adrenocorticosteroids/ antagonists</u>
mechlorethamine	teniposide	prednisone and equivalents
cyclophosphamide	<u>Antibiotics</u>	dexamethasone
ifosfamide	actinomycin D	ainoglutethimide
melphalan	daunomycin (rubido-mycin)	<u>Progestins</u>
chlorambucil	doxorubicin (adria-mycin)	hydroxyprogesterone caproate
<u>Nitrosoureas</u>	mitoxantroneidarubicin	medroxyprogesterone acetate
carmustine (BCNU)	bleomycinpicamycin	megestrol acetate
lomustine (CCNU)	(mithramycin)	<u>Estrogens</u>
semustine (methyl-CCNU)	mitomycinC	diethylstilbestrol
<u>Ethylenimine/Methyl-melamine</u>	dactinomycin	ethynyl estradiol/ equivalents
thiethylenemelamine (TEM)	<u>Enzymes</u>	<u>Antiestrogen</u>
triethylene thiophosphoramidate	L-asparaginase	tamoxifen
(thiotepa)	<u>Biological response modifiers</u>	<u>Androgens</u>
hexamethylmelamine	interferon-alpha	testosterone propionate
(HMM, altretamine)	IL-2	fluoxymesterone/equivalents
<u>Alkyl sulfonates</u>	G-CSF	<u>Antiandrogens</u>
busulfan	GM-CSF	flutamide
<u>Triazines</u>	<u>Differentiation Agents</u>	gonadotropin-releasing
dacarbazine (DTIC)	retinoic acid derivatives	hormone analogs
<u>Antimetabolites</u>	<u>Radiosensitizers</u>	leuprolide
Folic Acid analogs	metronidazole	<u>Nonsteroidal antiandrogens</u>
methotrexate	misonidazole	flutamide
Trimetrexate	desmethylmisonidazole	<u>Photosensitizers</u>
Pemetrexed	pimonidazole	hematoporphyrin derivatives
Multi-targeted antifolate	etanidazole	Photofrin®
Pyrimidine analogs	nimorazole	benzoporphyrin derivatives
5-fluorouracil	RSU 1069	Npe6
fluorodeoxyuridine	EO9	tin etioporphyrin (SnET2)
gemcitabine	RB 6145	phorbolide-a
cytosine arabinoside	SR4233	bacteriochlorophyll-a
(AraC, cytarabine)	nicotinamide	naphthalocyanines
5-azacytidine	5-bromodeoxyuridine	phthalocyanines
2,2'- difluorodeoxy-cytidine	5-iododeoxyuridine	zinc phthalocyanines
Purine analogs	bromodeoxycytidine	
6-mercaptopurine	<u>Miscellaneous agents</u>	
6-thioguanine	<u>Platinum coordination complexes</u>	
azathioprine	cisplatin	
2'-deoxycoformycin	Carboplatin	
(pentostatin)	oxaliplatin	
erythrohydroxynonyl-adenine	<u>Anthracenedione</u>	
(EHNA)	mitoxantrone	
fludarabine phosphate	<u>Substituted urea</u>	
2-chlorodeoxyadenosine	hydroxyurea	
(cladribine, 2-CdA)	<u>Methylhydrazine derivatives</u>	
<u>Type I Topoisomerase</u>	N-methylhydrazine (MIH)	
<u>Inhibitors</u>	procarbazine	
camptothecin	<u>Adrenocortical suppressant</u>	
topotecan	mitotane (o,p'-DDD)	
irinotecan	ainoglutethimide	
<u>Natural products</u>	<u>Cytokines</u>	
<u>Antimitotic drugs</u>	interferon (*, *, *)	
paclitaxel	interleukin-2	
Vinca alkaloids		
vinblastine (VLB)		
vincristine		
vinorelbine		
Taxotere® (docetaxel)		
estramustine		
estramustine phosphate		

[00347] Cytokines that are effective in inhibiting tumor metastasis are also contemplated for use in the combination therapy. Such cytokines, lymphokines, or other hematopoietic factors include, but are not limited to, M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, TNF α , TNF1, TNF2, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin.

[00348] Alternatively, the therapeutic treatment employing a compound that inhibits binding between iASPP and an iASPP binding partner described herein or other therapeutic agent described herein may precede or follow the second agent treatment by intervals ranging from minutes to weeks. In embodiments where the second agent and the compound that inhibits binding between iASPP and an iASPP binding partner are administered separately, one would generally ensure that a significant period of time did not expire between the times of each delivery, such that the agent and the compound that inhibits binding between iASPP and an iASPP binding partner would still be able to exert an advantageously combined effect. In such instances, it is contemplated that one would administer both modalities within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations. Repeated treatments with one or both agents are specifically contemplated.

[00349] The term "treatment" includes combination treatments and therapies, in which two or more treatments or therapies are combined, for example, sequentially or simultaneously. For example, the compounds described herein may also be used in combination therapies, e.g., in conjunction with other agents, for example, cytotoxic agents, anticancer agents, etc. Examples of treatments and therapies include, but are not limited to, chemotherapy (the administration of active agents, including, e.g., drugs, antibodies (e.g., as in immunotherapy), prodrugs (e.g., as in photodynamic therapy, GDEPT, ADEPT, etc.); surgery; radiation therapy; photodynamic therapy; gene therapy; and controlled diets.

[00350] The particular combination would be at the discretion of the physician who would select dosages using his common general knowledge and dosing regimens known to a skilled practitioner.

[00351] The agents (e.g., a compound of the invention; plus one or more other agents) may be administered simultaneously or sequentially, and may be administered in individually varying dose schedules and via different routes. For example, when administered sequentially, the agents can be administered at closely spaced intervals (e.g., over a period of 5-10 minutes) or at longer intervals (e.g., 1, 2, 3, 4 or more hours apart, or even longer periods apart where required), the precise dosage regimen being commensurate with the properties of the therapeutic agent(s).

[00352] The agents may be formulated together in a single dosage form, or alternatively, the individual agents may be formulated separately and presented together in the form of a kit, optionally with instructions for their use, as described below.

[00353] In one embodiment, the patient may be suffering from a cancer condition in which iASPP is over-expressed. In another embodiment, the patient may be suffering from a cancer condition in which wild-type p53 function is present in at least a portion of the tumour cells.

[00354] In one aspect, the patient may be suffering from a breast cancer in which iASPP is over-expressed. This may be against a wild-type or non-wild-type p53 background.

[00355] In another aspect, the patient may be suffering from leukaemia in which iASPP is over-expressed. This may be against a wild-type or non-wild-type p53 background.

[00356] In a further embodiment, the treatment of the patient may be preceded by the step of determining on a sample of a tumour present in the patient, whether or not at least a portion of the tumour cells have wild-type p53. Optionally the status (i.e. wild-type or mutant) of p63 and/or p73 may be determined.

[00357] Thus a further aspect of the present invention provides a method for preparing a medicament, pharmaceutical composition or drug, the method comprising:

[00358] (a) identifying or modifying a compound by a method of any one of the other aspects of the invention disclosed herein; (b) optimising the structure of the molecule; and (c) preparing a medicament, pharmaceutical composition or drug containing the optimised compound.

[00359] The above-described processes of the invention may be iterated in that the modified compound may itself be the basis for further compound design.

[00360] By “optimising the structure” we mean e.g. adding molecular scaffolding, adding or varying functional groups, or connecting the molecule with other molecules (e.g. using a fragment linking approach) such that the chemical structure of the modulator molecule is changed while its original modulating functionality is maintained or enhanced. Such optimisation is regularly undertaken during drug development programmes to e.g. enhance potency, promote pharmacological acceptability, increase chemical stability etc. of lead compounds.

[00361] Modification will be those conventional in the art known to the skilled medicinal chemist, and will include, for example, substitutions or removal of groups containing residues which interact with the amino acid side chain groups of a iASPP C-terminal structure of the invention. For example, the replacements may include the addition or removal of groups in order to decrease or increase the charge of a group in a test compound, the replacement of a charge group with a group of the opposite charge, or the replacement of a hydrophobic group with a hydrophilic group or vice versa. It will be understood that these are only examples of the type of substitutions considered by medicinal chemists in the development of new pharmaceutical compounds and other modifications may be made, depending upon the nature of the starting compound and its activity.

Formulations and Routes of Administration

[00362] In order to prepare peptide-containing compositions for clinical use, it will be necessary to prepare the therapeutic peptides of the present invention as pharmaceutical compositions, *i.e.*, in a form appropriate for *in vivo* applications. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

[00363] One will generally desire to employ appropriate salts and buffers to render delivery vectors stable and allow for uptake by target cells. Buffers also will be employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention comprise an effective amount of the peptide or an expression vector to cells, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrase “pharmaceutically acceptable” or “pharmacologically acceptable” refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein,

“pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the vectors or cells of the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

[00364] The compositions of the present invention include classic pharmaceutical preparations. Administration of these compositions according to the present invention will be via any common route so long as the target tissue is available via that route. The pharmaceutical compositions may be introduced into the subject by any conventional method, *e.g.*, by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary (*e.g.*, term release); by oral, sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site. The treatment may consist of a single dose or a plurality of doses over a period of time.

[00365] The compositions may be prepared for administration as solutions of free base or pharmacologically acceptable salts in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00366] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00367] Sterile injectable solutions are prepared by incorporating the composition in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00368] For oral administration the peptides of the present invention may be incorporated with excipients and used in the form of non-ingestible mouthwashes and dentifrices. A mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an antiseptic wash containing sodium borate, glycerin and potassium bicarbonate. The active ingredient may also be dispersed in dentifrices, including: gels, pastes, powders and slurries. The active ingredient may be added in a therapeutically effective amount to a paste dentifrice that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants.

[00369] The compositions described herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[00370] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the

solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

[00371] “Unit dose” is defined as a discrete amount of a therapeutic composition dispersed in a suitable carrier. For example, where polypeptides are being administered parenterally, the polypeptide compositions are generally injected in doses ranging from 1 µg/kg to 100mg/kg body weight/day, preferably at doses ranging from 0.1mg/kg to about 50 mg/kg body weight/day. Parenteral administration may be carried out with an initial bolus followed by continuous infusion to maintain therapeutic circulating levels of drug product. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient.

[00372] The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the routes of administration. The optimal pharmaceutical formulation will be determined by one of skill in the art depending on the route of administration and the desired dosage. See for example Remington’s Pharmaceutical Sciences, 18th Ed. (1990, Mack Publ. Co, Easton PA 18042) pp 1435-1712, incorporated herein by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release and rate of *in vivo* clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface areas or organ size. Further refinement of the calculations necessary to determine the appropriate treatment dose is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein as well as the pharmacokinetic data observed in animals or human clinical trials.

[00373] Appropriate dosages may be ascertained through the use of established assays for determining blood clotting levels in conjunction with relevant dose-response data. The final dosage regimen will be determined by the attending physician, considering factors that modify the action of drugs, *e.g.*, the drug’s specific activity, severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding appropriate dosage levels and duration of treatment for specific diseases and conditions.

[00374] In gene therapy embodiments employing viral delivery, the unit dose may be calculated in terms of the dose of viral particles being administered. Viral doses include a particular number of virus particles or plaque forming units (pfu). For embodiments involving adenovirus, particular unit doses include 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} or 10^{14} pfu. Particle doses may be somewhat higher (10 to 100-fold) due to the presence of infection defective particles.

[00375] It will be appreciated that the pharmaceutical compositions and treatment methods described herein may be useful in fields of human medicine and veterinary medicine. Thus the subject to be treated may be a mammal, (e.g., human) or other animal. For veterinary purposes, subjects include for example, farm animals including cows, sheep, pigs, horses and goats, companion animals such as dogs and cats, exotic and/or zoo animals, laboratory animals including mice rats, rabbits, guinea pigs and hamsters; and poultry such as chickens, turkey ducks and geese.

[00376] The invention is illustrated by the following examples:

Examples

Summary of Examples

[00377] The expression levels of ASPP proteins are altered in tumours. Whilst ASPP1 and ASPP2 are down regulated in a large percentage of tumours (Agirre et al., 2006; Bergamaschi et al., 2003; Liu et al., 2004; Lossos et al., 2002; Samuels-Lev et al., 2001), iASPP is up regulated in human breast carcinomas expressing wild-type p53 (Bergamaschi et al., 2003). iASPP is also overexpressed in acute leukemias regardless of p53 mutation status (Zhang et al., 2005), suggesting that iASPP may promote carcinogenesis by other mechanisms additional to p53 inhibition. As iASPP is functionally distinct from the other family members, and an inhibitor of p53 dependent apoptosis up regulated in cancer, a strategy based on antagonising the ability of iASPP to bind p53 could be an important target for treating cancer. With the exception of the well characterised ASPP2-p53 interaction (Gorina and Pavletich, 1996; Tidow et al., 2006) there is a paucity of information at the molecular level on the interactions of these protein families.

[00378] A solid phase binding assay (Example 1) revealed that the C-terminal region of iASPP (residues 625-828, named iASPP Δ 625), like its homologues, is able to interact with the DNA binding domains of p63 and p73. However, there were significant differences in the equilibrium binding constants for iASPP Δ 625 and the homologous region in ASPP2. To further

characterise the p53 binding site of iASPP we determined a high resolution (2.1 Å) crystal structure of the iASPP C-terminal domain (residues 608-828, named iASPPΔ608) - see Example 2. A fortuitous lattice packing in the iASPPΔ608 crystals revealed that the N-terminal residues of one molecule can interact with the putative p53 binding site of a second molecule.

[00379] Further studies set out in Example 3 using isothermal titration calorimetry (ITC) confirmed that an 8-mer iASPP peptide corresponding to residues 616-623 can indeed bind the C-terminal domain of iASPP with a binding affinity comparable to previously measured SH3-peptide interactions (Dalgarno et al., 1997)..

Example 1. Differential binding of iASPP and ASPP2 to the p53 family

[00380] We investigated whether the C-terminal domains of iASPP and ASPP2; iASPP residues 625-828 (iASPPΔ625) and ASPP2 residues 905-1128 (ASPP2Δ905) bind the core DNA binding domains of p53, p63 and p73; p53 residues 94-292 (p53_{core}), p63 residues 123-323 (p63_{core}), and p73 residues 112-312 (p73_{core}), with varying affinities using a solid phase binding assay.

[00381] For solid phase binding experiments two ASPP constructs; iASPPΔ625 and ASPP2Δ905 encoding iASPP residues 625-628 and ASPP2 residues 905-1128, respectively were made using the bacterial expression vector pET22b (Novagen). Nucleotides encoding the required amino acids were amplified by PCR introducing a hexa-histidine sequence in 5' and a stop codon in 3', cloned into the expression vector using NdeI and EcoRI restriction sites and sequenced. Constructs encoding the DNA binding domain of p53 residues 94-292 (p53_{core}), p63 residues 123-323 (p63_{core}) and p73 residues 112-312 (p73_{core}), used in solid phase binding assays, were also made using pET22b essentially as for ASPP constructs but without the incorporation of a hexa-histidine coding sequence.

[00382] ASPP proteins were expressed in the *E.coli* strain Rosetta pLysS (Invitrogen), bacteria were grown in Luria broth (LB) with 100 µg/ml ampicillin and protein expression induced by addition of 0.25 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Bacterial pellets were suspended in ice cold high salt PBS (500 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄/NaH₂PO₄ pH 8.0), lysed by sonication and clarified by centrifugation. Recombinant proteins were purified from the soluble fraction by Ni²⁺ affinity chromatography then further purified by size exclusion chromatography into the appropriate buffer. The recombinant proteins

p53_{core}, p63_{core} and p73_{core} were purified as previously described for the DNA binding domain of p53 (Derbyshire et al., 2002).

[00383] Purified iASPPΔ625, ASPP2Δ905, p53_{core}, p63_{core} and p73_{core} were buffer exchanged by size exclusion chromatography into PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄/NaH₂PO₄ pH 7.4). The DNA binding domains of p53, p63 and p73 were immobilised on 96-well microtiter plates (Nunc, PolySorp), 10 pM per well, over-night at 4 °C. Remaining binding sites were saturated by one hour incubation at 4 °C in PBS containing 1% bovine serum albumin (BSA). Microtiter plates were incubated for one hour at 4 °C with a dilution series of either iASPPΔ625 or ASPP2Δ905 then incubated with 1/1000 dilution of monoclonal antibody solution (anti-iASPP LX142.1 [raised against iASPPΔ625] or anti-ASPP2 LX141.2 [raised against ASPP2Δ905]), plates were then probed for one hour at 4 °C with an anti-mouse IgG alkaline phosphatase conjugate antibody (Sigma-Aldrich). The bound alkaline phosphatase conjugate antibody was detected by the addition of the substrate *p*-Nitrophenyl Phosphate. Progress curves were recorded for forty minutes at room temperature, at 405 nm, using a Thermo Labsystems Multiskan Ascent microplate reader and the initial rates were determined using the SigmaPlot 10 software (Systat Software Inc.). Each data point represents the average of duplicate wells, ± the standard deviation. Non specific binding was determined from iASPPΔ625 or ASPP2Δ905 bound to BSA and was subtracted from each data point.

[00384] Data points were plotted, rate term against ligand concentration, resulting in saturation curves. Scatchard analysis was also undertaken. In order to determine K_d values, saturation curves were fitted by non-linear regression analysis using the SigmaPlot 10 software.

[00385] Non linear regression fitting of saturation curves revealed that iASPPΔ625 and ASPP2Δ905 bind the DNA binding domains all three p53 family members (Figure 1). Both iASPPΔ625 and ASPP2Δ905 bind p53_{core} with a similar high affinity, equilibrium dissociation constants (K_d values) measured were 26.4 ± 1.7 nM and 23.3 ± 1.6 nM respectively. Values measured for iASPPΔ625 and ASPP2Δ905 binding p63_{core} and p73_{core} showed significant differences; iASPPΔ625 binds p63_{core} and p73_{core} with an affinity approximately three fold higher than that of ASPP2Δ905 and both iASPPΔ625 and ASPP2Δ905 have a three fold higher affinity for p63_{core} over p73_{core}. K_d values for iASPPΔ625 and ASPP2Δ905 binding p63_{core} were 128.1 ± 11 nM and 343.1 ± 39.8 nM respectively and for p73_{core}, 331.3 ± 66 nM and 984.4 ± 196.1 nM respectively.

Example 2. Crystal Structure of the C-terminal region of iASPP

[00386] The crystal structure of the C-terminal domain of iASPP residues 608-828 (iASPP Δ 608), was determined by molecular replacement and refined to 2.1 Å resolution. For crystallization experiments the construct iASPP Δ 608 encoding human iASPP residues 608-828 of SEQ ID NO: 8 was made using the same strategy as for the other ASPP constructs as described in Example 1.

[00387] Prior to crystallization iASPP Δ 608 was concentrated by ultrafiltration in size exclusion buffer (150 mM NaCl, 25 mM Tris pH 8.0, 1 mM DTT) to 8 mg/ml. Sitting drop vapour diffusion crystallization trials (drop size: 100 nl protein plus 100 nl reservoir solution) were set up with a Cartesian robot as previously reported (Brown et al., 2003; Walter et al., 2003). iASPP Δ 608 initially crystallized at 20 °C out of a mother liquor containing 20% (w/v) PEG 3350, 0.2 M potassium chloride (Hampton Peg/Ion screen, reagent 8). Optimised crystals grow at 20 °C out of a mother liquor containing 22 % (w/v) PEG 3350, 0.3 M potassium chloride and 17.5 % MPD. The crystals cryo-protected in mother liquor diffracted to 2.1 Å after flash-freezing at 100 K on beamline ID14EH2 at the ESRF. X-ray data were processed and scaled with the HKL suite (Otwinowski and Minor, 1997). The program XPREP (www.bruker-nonline.com) was used to calculate quality indicators and to merge data. Crystallographic statistics are shown in Table 11.

Table 11. Crystallographic statistics

Data collection	
Beamline	ESRF-ID14EH2
Resolution	30.0-2.1 (2.2-2.1)
Spacegroup	P2 ₁ 2 ₁ 2
Cell dimensions (Å)	a = 60.0 b = 67.4 c = 50.5
Wavelength (Å)	0.9757
Unique reflection	12492 (1197)
Completeness (%)	99.8 (98.3)
R _{merge} (%) ^a	11.7 (76.5)
I/ σ I	13.3 (1.5)
Average redundancy	4.4 (2.8)
Refinement	
Resolution range (Å)	30.0-2.1
Number of reflections	12486
R _{factor} (%) ^b	20.7
R _{free} (%) ^c	25.2
r.m.s.d. bonds(Å)	0.008
r.m.s.d. angles(deg)	1.365

r.m.s.d. main-chain bond B(Å ²)	1.394
r.m.s.d. side-chain bond B(Å ²)	2.342
No. of atoms per asymmetric unit (protein/water)	1592/154
Average B factors (protein/water) (Å ²)	26.7/41.2

[00388] Numbers in parentheses refer to the appropriate outer shell. r.m.s.d.: root mean square deviation from ideal geometry.

[00389] $^a R_{\text{merge}} = \sum_{\text{hkl}} \sum_i |I(\text{hkl}; i) - \langle I(\text{hkl}) \rangle| / \sum_{\text{hkl}} \sum_i I(\text{hkl}; i)$, where $I(\text{hkl}; i)$ is the intensity of an individual measurement and $\langle I(\text{hkl}) \rangle$ is the average intensity from multiple observations.

[00390] $^b R_{\text{factor}} = \sum_{\text{hkl}} ||F_{\text{obs}}| - k|F_{\text{calc}}|| / \sum_{\text{hkl}} |F_{\text{obs}}|$

[00391] $^c R_{\text{free}}$ equals the R -factor against 5% of the data removed prior to refinement.

[00392] The iASPPΔ608 crystal structure was determined using the molecular replacement method. Real space cross-rotation function, PC refinement of the rotation peaks, and fast F2F2 translation searches within a data range of 15-3 Å with the program CNS (Brünger et al., 1998) using the structure coordinates of ASPP2 (PDB ID code 1YCS, sequence identity: 53%) as the search model resulted in the correct orientation and position of the iASPP monomer in the crystallographic asymmetric unit. Refinement was carried out using program CNS by iterative cycles of simulated annealing, conjugate gradient minimization, individual B-factor refinement and manual rebuilding using program O (Jones et al., 1991). Final refinement resulted in an R -factor (R_{free}) of 20.7 % (25.2 %) (see also crystallographic statistics in Table 11). The stereochemical properties of the structure were assessed by PROCHECK (Laskowski et al., 1993) and WHATCHECK (Hooft et al., 1996) and showed no residue in disallowed regions of the Ramachandran plot. Structural superpositions were made using the program SHP (Stuart et al., 1979) and all figures were produced using Pymol (www.pymol.org).

[00393] The iASPPΔ608 crystal structure shows that the C-terminal domain of iASPP is made up of four ankyrin repeats and a closely juxtaposed SH3 domain that form a single structural unit with a significant buried surface area between the two protein motifs. The close proximity of the ankyrin repeats and the SH3 domain is a characteristic feature of ASPP proteins, indeed, so far only ASPP proteins have been shown to adopt a structural unit consisting of ankyrin repeats closely juxtaposed to an SH3 domain. Without structural information it is difficult to determine whether this domain arrangement is present in other proteins, as terminal ankyrin

repeats usually deviate from the established consensus sequence and are often not recognised (Mosavi et al., 2004). However, the proteins CASKIN1 and CASKIN2 (Tabuchi et al., 2002) may contain a similar structural arrangement to that observed in the crystal structures of the C-terminal domains of iASPP and ASPP2. They are predicted to have six ankyrin repeats, then a 34 residue linker (the approximate average length of an ankyrin repeat) whose sequence is predicted to contain two helices, followed by an SH3 domain. The amino acid sequences which make up the C-terminal ankyrin repeat of iASPP and ASPP2 constitute two α -helices and lack a β -hairpin loop.

[00394] The data show that iASPP Δ 625 and ASPP2 Δ 905 have a similar high affinity for p53_{core}, the K_d value determined for the ASPP2 Δ 905- p53_{core} interaction in our solid state binding assay (23 nM) is very similar to results obtained by (Gorina and Pavletich, 1996) using surface plasmon resonance (30 nM). iASPP Δ 625 is able to interact with p63_{core} and p73_{core} with a three fold higher affinity than ASPP2 Δ 905, raising the possibility that iASPP, like ASPP2, plays a role in the regulation of p63 and p73 dependent apoptosis. ASPP1 and ASPP2 have been shown to bind all p53 family members and stimulate the transactivation of function of p53, p63 and p73 on the promoters of apoptotic genes (Bergamaschi et al., 2004; Samuels-Lev et al., 2001). Our data now provide comparable biochemical affinity measurements for iASPP and ASPP2 binding p53, p63 and p73. Relative to functional data, analysis of our crystal structure suggests some reasons why compared to ASPP2 Δ 905, iASPP Δ 625 has an approximate three fold greater affinity for p63_{core} and p73_{core}. In particular the SH3 domain of iASPP, unlike that of ASPP2, has a tyrosine (Tyr 814) that is likely to form classical interactions with the hydrophobic P₀ residue in the linear (peptide) motif of the L3 loop of p63 and p73. The iASPP Δ 608 crystal packing suggests that a peptide based on the sequence of iASPP residues 616-623 would have the appropriate properties to bind to the C-terminal part of iASPP. This peptide binding site overlaps the putative binding site on iASPP for the DNA binding domain of p53 and thus such peptides (or polypeptide sequences) would be predicted to compete with p53 for iASPP binding.

Example 3: Binding of the P1 peptide

[00395] To further characterize the binding properties of the iASPP Δ 608 N-terminus by isothermal titration calorimetry (ITC), the construct iASPP Δ 625 lacking the interacting N-terminal residues was used and a peptide synthesized comprising iASPP residues 616-623 (peptide P1).

[00396] The P1 peptide (SPRKARRA; SEQ ID NO:2) was synthesized using standard technology and purified by high-pressure liquid chromatography (ALTA Bioscience, Birmingham). Experiments were performed using a VP-ITC microcalorimeter (MicroCal, Northampton, MA). Purified iASPPΔ625 was dialysed into a buffer consisting of 50 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄/NaH₂PO₄ pH 8.0. The dialysis buffer was also used to dissolve the peptide. At a concentration of 1.5 mM P1 was titrated from a syringe (300 μl total volume) into a sample cell containing 1.4 ml iASPPΔ625 (0.1 mM). Prior to experiments both peptide and protein solutions were clarified for 10 min at 16100 g and degassed (ThermoVac; MicroCal). At the equilibrium temperature of 15°C, peptide P1 was titrated into iASPPΔ625 by 44 injections of 5 μl each. Resulting peaks of measured deviations from the equilibrium temperature were integrated to yield the quantity of heat generated. The control experiment; injecting the peptide at the above concentration into buffer alone, using the same experimental conditions, to find the heat generated by peptide dilution, was performed and the integrated data were subtracted from peptide into protein data. Heat generated by protein dilution was determined by injecting buffer alone into a sample chamber filled with iASPPΔ625 and was found to be negligible. Data were fitted using χ^2 minimization on a model assuming a single set of binding sites to calculate the dissociation constant, K_d . All steps of the data analysis were performed using ORIGIN (V7.0) software provided by MicoCal.

[00397] As illustrated in Figure 5, the results indicate that each iASPP molecule binds one peptide molecule with an estimated K_d of 45 μM. This is the average of three separate experiments.

Sequences

[00398] SEQ ID NO:1 - Fragment of iASPP. Residues 8 to 228 of SEQ ID NO:1 correspond to amino acids 608-828 of iASPP (NP_006654, SEQ ID NO: 8). Residues 2-7 are a hexahistidine tag.

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MHHHHHRSV LRKAGSPRKA RRARLNPLVL LLDAALTGEL EVVQQAVKEM NDPSQPNEEG 60
ITALHNAICG ANYSIVDFLI TAGANVNSPD SHGWTPLHCA ASCNDTVICM ALVQHGA AIF 120
ATTLSDGATA FEKCDPYREG YADCATYLAD VEQSMGLMNS GAVYALWDYS AEFGD ELSFR 180
EGESVTVLRR DGPEETDWWW AALHGQEGYV PRNYFGLFPR VKPQR SKV 228
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[00399] SEQ ID NO:2 - Peptide P1 - SPRKARRA

[00400] SEQ ID NO:3 - Peptide P1 Ala5Ser - SPRKSRRRA

[00401] SEQ ID NO:4 - Peptide P1 Lys4Arg - SPRRARRA

[00402] SEQ ID NO:5 - Peptide P1 Lys4Arg+Ala5Ser - SPRRSRRA

[00403] SEQ ID NO: 6- $X_1X_2X_3X_4X_5X_6X_7X_8$, wherein X_1 is selected from Serine or Threonine or conservative substitution thereof; X_2 is proline or conservative substitution thereof; X_3 , X_4 , X_6 , and X_7 are each independently selected from Arginine and Lysine; X_5 is Alanine, Serine, Valine, Glycine or conservative substitutions thereof; and X_8 is Alanine, Valine, Glycine or conservative substitutions thereof;

[00404] SEQ ID NO 7 - full-length iASPP polynucleotide sequence

[00405] SEQ ID NO: 8 - full-length iASPP amino acid sequence

[00406] References:

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[00458] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described invention will be apparent to those of skill in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

Table 1

Table 1																			
ATOM	1	CB	SER A 616	53.794	39.136	16.540	1.00	57.10	A	ATOM	49	O	ARG A 621	44.775	29.682	7.070	1.00	25.09	A
ATOM	2	OG	SER A 616	53.817	37.719	16.487	1.00	57.91	A	ATOM	50	N	ARG A 622	46.792	28.694	7.053	1.00	27.11	A
ATOM	3	C	SER A 616	51.315	39.090	16.172	1.00	55.04	A	ATOM	51	CA	ARG A 622	46.715	28.342	5.645	1.00	26.30	A
ATOM	4	O	SER A 616	51.306	38.075	16.874	1.00	56.21	A	ATOM	52	CB	ARG A 622	48.061	27.799	5.160	1.00	24.39	A
ATOM	5	N	SER A 616	52.613	41.198	15.837	1.00	58.37	A	ATOM	53	CG	ARG A 622	49.154	28.864	5.083	1.00	23.02	A
ATOM	6	CA	SER A 616	52.635	39.712	15.713	1.00	56.56	A	ATOM	54	CD	ARG A 622	48.893	29.858	3.945	1.00	22.30	A
ATOM	7	N	PRO A 617	50.178	39.687	15.777	1.00	52.62	A	ATOM	55	NE	ARG A 622	48.998	29.241	2.621	1.00	22.11	A
ATOM	8	CD	PRO A 617	49.986	40.796	14.828	1.00	51.51	A	ATOM	56	CZ	ARG A 622	50.146	28.898	2.039	1.00	24.40	A
ATOM	9	CA	PRO A 617	48.886	39.135	16.185	1.00	50.72	A	ATOM	57	NH1	ARG A 622	51.300	29.116	2.662	1.00	24.43	A
ATOM	10	CB	PRO A 617	47.903	40.190	15.704	1.00	50.24	A	ATOM	58	NH2	ARG A 622	50.144	28.322	0.840	1.00	22.17	A
ATOM	11	CG	PRO A 617	48.523	40.630	14.430	1.00	50.85	A	ATOM	59	C	ARG A 622	45.630	27.256	5.601	1.00	27.49	A
ATOM	12	C	PRO A 617	48.635	37.784	15.523	1.00	49.63	A	ATOM	60	O	ARG A 622	45.622	26.332	6.419	1.00	26.07	A
ATOM	13	O	PRO A 617	49.389	37.351	14.651	1.00	49.15	A	ATOM	61	N	ALA A 623	44.710	27.393	4.654	1.00	28.64	A
ATOM	14	N	ARG A 618	47.568	37.123	15.947	1.00	48.77	A	ATOM	62	CA	ALA A 623	43.575	26.487	4.489	1.00	30.34	A
ATOM	15	CA	ARG A 618	47.203	35.831	15.392	1.00	48.24	A	ATOM	63	CB	ALA A 623	42.866	26.814	3.172	1.00	29.28	A
ATOM	16	CB	ARG A 618	46.226	35.131	16.334	1.00	49.76	A	ATOM	64	C	ALA A 623	43.785	24.970	4.583	1.00	30.64	A
ATOM	17	CG	ARG A 618	45.037	35.998	16.692	1.00	50.37	A	ATOM	65	O	ALA A 623	42.901	24.263	5.070	1.00	31.57	A
ATOM	18	CD	ARG A 618	44.252	35.435	17.858	1.00	51.92	A	ATOM	66	N	ARG A 624	44.924	24.457	4.127	1.00	29.65	A
ATOM	19	NE	ARG A 618	43.247	36.389	18.315	1.00	53.49	A	ATOM	67	CA	ARG A 624	45.131	23.006	4.156	1.00	31.33	A
ATOM	20	CZ	ARG A 618	42.196	36.772	17.598	1.00	54.56	A	ATOM	68	CB	ARG A 624	45.742	22.544	2.822	1.00	32.73	A
ATOM	21	NH1	ARG A 618	41.999	36.275	16.383	1.00	54.45	A	ATOM	69	CG	ARG A 624	44.880	22.926	1.609	1.00	36.92	A
ATOM	22	NH2	ARG A 618	41.351	37.671	18.090	1.00	55.09	A	ATOM	70	CD	ARG A 624	45.438	22.420	0.278	1.00	40.90	A
ATOM	23	C	ARG A 618	46.551	36.049	14.030	1.00	46.43	A	ATOM	71	NE	ARG A 624	45.445	20.959	0.194	1.00	43.89	A
ATOM	24	O	ARG A 618	46.025	37.123	13.747	1.00	46.77	A	ATOM	72	CZ	ARG A 624	46.513	20.192	0.416	1.00	45.35	A
ATOM	25	N	LYS A 619	46.594	35.032	13.184	1.00	44.20	A	ATOM	73	NH1	ARG A 624	47.688	20.735	0.731	1.00	42.70	A
ATOM	26	CA	LYS A 619	45.992	35.132	11.867	1.00	41.92	A	ATOM	74	NH2	ARG A 624	46.397	18.873	0.347	1.00	45.03	A
ATOM	27	CB	LYS A 619	47.011	35.645	10.846	1.00	42.27	A	ATOM	75	C	ARG A 624	45.943	22.451	5.323	1.00	30.16	A
ATOM	28	CG	LYS A 619	48.434	35.150	11.053	1.00	43.74	A	ATOM	76	O	ARG A 624	46.297	21.271	5.330	1.00	29.71	A
ATOM	29	CD	LYS A 619	48.625	33.705	10.654	1.00	43.51	A	ATOM	77	N	LEU A 625	46.238	23.292	6.308	1.00	29.28	A
ATOM	30	CE	LYS A 619	50.087	33.308	10.806	1.00	44.20	A	ATOM	78	CA	LEU A 625	47.001	22.848	7.471	1.00	29.06	A
ATOM	31	NZ	LYS A 619	50.331	31.904	10.388	1.00	45.34	A	ATOM	79	CB	LEU A 625	47.851	23.995	8.022	1.00	29.36	A
ATOM	32	C	LYS A 619	45.456	33.788	11.431	1.00	40.21	A	ATOM	80	CG	LEU A 625	48.939	24.506	7.085	1.00	31.00	A
ATOM	33	O	LYS A 619	45.904	32.748	11.918	1.00	38.96	A	ATOM	81	CD1	LEU A 625	49.681	25.662	7.737	1.00	32.96	A
ATOM	34	N	ALA A 620	44.482	33.823	10.524	1.00	37.27	A	ATOM	82	CD2	LEU A 625	49.891	23.368	6.757	1.00	32.52	A
ATOM	35	CA	ALA A 620	43.883	32.607	10.002	1.00	34.76	A	ATOM	83	C	LEU A 625	46.054	22.347	8.557	1.00	26.96	A
ATOM	36	CB	ALA A 620	42.792	32.941	9.005	1.00	34.96	A	ATOM	84	O	LEU A 625	44.967	22.889	8.739	1.00	28.04	A
ATOM	37	C	ALA A 620	44.973	31.783	9.335	1.00	32.67	A	ATOM	85	N	ASN A 626	46.466	21.316	9.280	1.00	25.67	A
ATOM	38	O	ALA A 620	45.778	32.295	8.559	1.00	32.56	A	ATOM	86	CA	ASN A 626	45.622	20.772	10.335	1.00	25.73	A
ATOM	39	N	ARG A 621	44.983	30.500	9.661	1.00	30.59	A	ATOM	87	CB	ASN A 626	46.000	19.310	10.587	1.00	28.43	A
ATOM	40	CA	ARG A 621	45.953	29.545	9.145	1.00	28.88	A	ATOM	88	CG	ASN A 626	45.834	18.452	9.332	1.00	31.90	A
ATOM	41	CB	ARG A 621	45.767	28.205	9.851	1.00	27.88	A	ATOM	89	OD1	ASN A 626	44.788	18.493	8.683	1.00	34.21	A
ATOM	42	CG	ARG A 621	44.378	27.616	9.638	1.00	28.84	A	ATOM	90	ND2	ASN A 626	46.863	17.685	8.981	1.00	31.40	A
ATOM	43	CD	ARG A 621	44.322	26.154	10.055	1.00	35.63	A	ATOM	91	C	ASN A 626	45.762	21.628	11.591	1.00	25.10	A
ATOM	44	NE	ARG A 621	43.042	25.523	9.733	1.00	37.32	A	ATOM	92	O	ASN A 626	46.844	21.744	12.162	1.00	24.06	A
ATOM	45	CZ	ARG A 621	41.913	25.699	10.417	1.00	38.52	A	ATOM	93	N	PRO A 627	44.657	22.257	12.026	1.00	25.02	A
ATOM	46	NH1	ARG A 621	41.888	26.494	11.483	1.00	37.94	A	ATOM	94	CD	PRO A 627	43.306	22.150	11.443	1.00	25.02	A
ATOM	47	NH2	ARG A 621	40.805	25.074	10.031	1.00	37.38	A	ATOM	95	CA	PRO A 627	44.647	23.120	13.212	1.00	24.95	A
ATOM	48	C	ARG A 621	45.786	29.312	7.656	1.00	26.51	A	ATOM	96	CB	PRO A 627	43.178	23.541	13.327	1.00	26.69	A
										ATOM	97	CG	PRO A 627	42.426	22.440	12.625	1.00	26.59	A
										ATOM	98	C	PRO A 627	45.204	22.542	14.516	1.00	23.78	A
										ATOM	99	O	PRO A 627	45.859	23.256	15.264	1.00	22.18	A

ATOM	100	N	LEU A 628	44.960	21.265	14.794	1.00	22.91	A	ATOM	151	O	ALA A 634	53.021	27.472	21.489	1.00	25.85	A
ATOM	101	CA	LEU A 628	45.466	20.673	16.032	1.00	23.00	A	ATOM	152	N	ALA A 635	51.970	25.495	21.327	1.00	25.65	A
ATOM	102	CB	LEU A 628	44.917	19.254	16.211	1.00	21.90	A	ATOM	153	CA	ALA A 635	52.063	25.240	22.758	1.00	26.12	A
ATOM	103	CG	LEU A 628	45.356	18.530	17.497	1.00	22.09	A	ATOM	154	CB	ALA A 635	51.096	24.138	23.169	1.00	24.43	A
ATOM	104	CD1	LEU A 628	44.866	19.307	18.722	1.00	20.85	A	ATOM	155	C	ALA A 635	53.488	24.847	23.112	1.00	26.58	A
ATOM	105	CD2	LEU A 628	44.802	17.107	17.505	1.00	20.92	A	ATOM	156	O	ALA A 635	53.970	25.152	24.196	1.00	29.25	A
ATOM	106	C	LEU A 628	46.996	20.642	16.038	1.00	24.66	A	ATOM	157	N	LEU A 636	54.158	24.180	22.179	1.00	28.35	A
ATOM	107	O	LEU A 628	47.636	20.862	17.077	1.00	24.69	A	ATOM	158	CA	LEU A 636	55.529	23.724	22.373	1.00	30.06	A
ATOM	108	N	VAL A 629	47.575	20.376	14.872	1.00	24.19	A	ATOM	159	CB	LEU A 636	55.854	22.631	21.354	1.00	30.25	A
ATOM	109	CA	VAL A 629	49.022	20.324	14.731	1.00	24.32	A	ATOM	160	CG	LEU A 636	57.227	21.964	21.431	1.00	31.43	A
ATOM	110	CB	VAL A 629	49.428	19.868	13.315	1.00	24.69	A	ATOM	161	CD1	LEU A 636	57.413	21.322	22.799	1.00	31.57	A
ATOM	111	CG1	VAL A 629	50.950	19.831	13.185	1.00	25.06	A	ATOM	162	CD2	LEU A 636	57.337	20.911	20.339	1.00	31.41	A
ATOM	112	CG2	VAL A 629	48.842	18.498	13.035	1.00	23.98	A	ATOM	163	C	LEU A 636	56.561	24.844	22.258	1.00	31.87	A
ATOM	113	C	VAL A 629	49.621	21.697	15.002	1.00	23.51	A	ATOM	164	O	LEU A 636	57.536	24.874	23.011	1.00	32.33	A
ATOM	114	O	VAL A 629	50.651	21.807	15.652	1.00	25.22	A	ATOM	165	N	THR A 637	56.345	25.764	21.319	1.00	33.16	A
ATOM	115	N	LEU A 630	48.970	22.742	14.508	1.00	24.32	A	ATOM	166	CA	THR A 637	57.271	26.872	21.102	1.00	34.90	A
ATOM	116	CA	LEU A 630	49.457	24.099	14.714	1.00	24.89	A	ATOM	167	CB	THR A 637	57.325	27.272	19.609	1.00	35.34	A
ATOM	117	CB	LEU A 630	48.659	25.087	13.862	1.00	24.79	A	ATOM	168	OG1	THR A 637	56.061	27.808	19.205	1.00	35.24	A
ATOM	118	CG	LEU A 630	48.648	24.823	12.353	1.00	28.30	A	ATOM	169	CG2	THR A 637	57.650	26.057	18.747	1.00	34.54	A
ATOM	119	CD1	LEU A 630	47.906	25.945	11.642	1.00	28.49	A	ATOM	170	C	THR A 637	56.950	28.108	21.940	1.00	36.14	A
ATOM	120	CD2	LEU A 630	50.064	24.723	11.830	1.00	29.15	A	ATOM	171	O	THR A 637	57.834	28.918	22.209	1.00	37.41	A
ATOM	121	C	LEU A 630	49.335	24.466	16.189	1.00	25.66	A	ATOM	172	N	GLY A 638	55.688	28.268	22.333	1.00	37.17	A
ATOM	122	O	LEU A 630	50.170	25.195	16.730	1.00	23.17	A	ATOM	173	CA	GLY A 638	55.326	29.397	23.174	1.00	38.31	A
ATOM	123	N	LEU A 631	48.283	23.957	16.828	1.00	26.06	A	ATOM	174	C	GLY A 638	54.471	30.526	22.631	1.00	39.68	A
ATOM	124	CA	LEU A 631	48.045	24.207	18.243	1.00	25.83	A	ATOM	175	O	GLY A 638	54.484	31.625	23.190	1.00	40.73	A
ATOM	125	CB	LEU A 631	46.722	23.576	18.687	1.00	25.03	A	ATOM	176	N	GLU A 639	53.716	30.281	21.568	1.00	40.44	A
ATOM	126	CG	LEU A 631	46.469	23.522	20.205	1.00	26.99	A	ATOM	177	CA	GLU A 639	52.872	31.329	21.003	1.00	40.62	A
ATOM	127	CD1	LEU A 631	46.528	24.933	20.805	1.00	25.58	A	ATOM	178	CB	GLU A 639	52.788	31.155	19.488	1.00	42.97	A
ATOM	128	CD2	LEU A 631	45.107	22.880	20.478	1.00	24.44	A	ATOM	179	CG	GLU A 639	54.140	31.325	18.785	1.00	47.22	A
ATOM	129	C	LEU A 631	49.176	23.617	19.069	1.00	25.66	A	ATOM	180	CD	GLU A 639	54.728	32.733	18.935	1.00	49.77	A
ATOM	130	O	LEU A 631	49.769	24.298	19.906	1.00	24.60	A	ATOM	181	OE1	GLU A 639	55.822	32.987	18.382	1.00	50.33	A
ATOM	131	N	LEU A 632	49.477	22.346	18.827	1.00	26.87	A	ATOM	182	OE2	GLU A 639	54.100	33.588	19.601	1.00	50.95	A
ATOM	132	CA	LEU A 632	50.527	21.671	19.580	1.00	28.11	A	ATOM	183	C	GLU A 639	51.478	31.343	21.638	1.00	40.15	A
ATOM	133	CB	LEU A 632	50.577	20.191	19.193	1.00	27.09	A	ATOM	184	O	GLU A 639	50.546	30.686	21.162	1.00	39.79	A
ATOM	134	CG	LEU A 632	49.298	19.402	19.490	1.00	27.20	A	ATOM	185	N	LEU A 640	51.357	32.122	22.713	1.00	38.38	A
ATOM	135	CD1	LEU A 632	49.522	17.924	19.192	1.00	26.76	A	ATOM	186	CA	LEU A 640	50.133	32.253	23.491	1.00	38.54	A
ATOM	136	CD2	LEU A 632	48.916	19.585	20.941	1.00	27.40	A	ATOM	187	CB	LEU A 640	50.367	33.223	24.644	1.00	38.35	A
ATOM	137	C	LEU A 632	51.900	22.323	19.395	1.00	29.25	A	ATOM	188	CG	LEU A 640	49.186	33.425	25.595	1.00	39.72	A
ATOM	138	O	LEU A 632	52.681	22.425	20.347	1.00	30.03	A	ATOM	189	CD1	LEU A 640	48.808	32.100	26.258	1.00	39.74	A
ATOM	139	N	ASP A 633	52.196	22.767	18.177	1.00	29.64	A	ATOM	190	CD2	LEU A 640	49.567	34.459	26.638	1.00	39.89	A
ATOM	140	CA	ASP A 633	53.471	23.425	17.928	1.00	30.74	A	ATOM	191	C	LEU A 640	48.852	32.657	22.769	1.00	40.36	A
ATOM	141	CB	ASP A 633	53.676	23.661	16.431	1.00	34.53	A	ATOM	192	O	LEU A 640	47.812	32.013	22.941	1.00	40.07	A
ATOM	142	CG	ASP A 633	53.728	22.362	15.639	1.00	40.46	A	ATOM	193	N	GLU A 641	48.908	33.728	21.985	1.00	41.51	A
ATOM	143	OD1	ASP A 633	54.322	21.385	16.146	1.00	42.50	A	ATOM	194	CA	GLU A 641	47.718	34.193	21.273	1.00	42.74	A
ATOM	144	OD2	ASP A 633	53.188	22.321	14.506	1.00	43.71	A	ATOM	195	CB	GLU A 641	48.026	35.466	20.474	1.00	45.24	A
ATOM	145	C	ASP A 633	53.510	24.754	18.677	1.00	29.63	A	ATOM	196	CG	GLU A 641	49.029	35.283	19.344	1.00	50.87	A
ATOM	146	O	ASP A 633	54.507	25.093	19.308	1.00	30.89	A	ATOM	197	CD	GLU A 641	50.329	34.661	19.813	1.00	54.91	A
ATOM	147	N	AIA A 634	52.412	25.499	18.618	1.00	27.80	A	ATOM	198	OE1	GLU A 641	50.905	35.155	20.809	1.00	56.65	A
ATOM	148	CA	AIA A 634	52.332	26.786	19.298	1.00	26.85	A	ATOM	199	OE2	GLU A 641	50.775	33.678	19.181	1.00	57.75	A
ATOM	149	CB	AIA A 634	51.006	27.464	18.978	1.00	25.18	A	ATOM	200	C	GLU A 641	47.186	33.102	20.349	1.00	41.11	A
ATOM	150	C	AIA A 634	52.474	26.609	20.805	1.00	25.99	A	ATOM	201	O	GLU A 641	45.972	32.928	20.219	1.00	40.28	A

ATOM	202	N	VAL A 642	48.097	32.368	19.713	1.00	38.70	A	ATOM	253	C	LYS A 648	37.367	28.648	20.427	1.00	39.84	A
ATOM	203	CA	VAL A 642	47.693	31.284	18.825	1.00	36.97	A	ATOM	254	O	LYS A 648	36.173	28.366	20.505	1.00	40.33	A
ATOM	204	CB	VAL A 642	48.926	30.614	18.161	1.00	37.58	A	ATOM	255	N	GLU A 649	38.116	28.329	19.378	1.00	39.48	A
ATOM	205	CG1	VAL A 642	48.493	29.426	17.305	1.00	36.37	A	ATOM	256	CA	GLU A 649	37.550	27.655	18.215	1.00	39.62	A
ATOM	206	CG2	VAL A 642	49.667	31.638	17.311	1.00	35.36	A	ATOM	257	CB	GLU A 649	38.519	27.747	17.036	1.00	41.41	A
ATOM	207	C	VAL A 642	46.915	30.258	19.656	1.00	34.25	A	ATOM	258	CG	GLU A 649	37.890	27.403	15.697	1.00	46.33	A
ATOM	208	O	VAL A 642	45.825	29.834	19.271	1.00	34.09	A	ATOM	259	CD	GLU A 649	38.918	27.251	14.592	1.00	49.64	A
ATOM	209	N	VAL A 643	47.477	29.874	20.801	1.00	31.80	A	ATOM	260	OE1	GLU A 649	39.859	28.076	14.530	1.00	51.44	A
ATOM	210	CA	VAL A 643	46.825	28.924	21.700	1.00	29.23	A	ATOM	261	OE2	GLU A 649	38.780	26.309	13.781	1.00	51.34	A
ATOM	211	CB	VAL A 643	47.655	28.713	22.990	1.00	28.39	A	ATOM	262	C	GLU A 649	37.207	26.191	18.471	1.00	37.20	A
ATOM	212	CG1	VAL A 643	46.850	27.902	23.997	1.00	26.12	A	ATOM	263	O	GLU A 649	36.121	25.730	18.127	1.00	37.04	A
ATOM	213	CG2	VAL A 643	48.964	28.012	22.662	1.00	24.37	A	ATOM	264	N	MET A 650	38.141	25.463	19.069	1.00	35.73	A
ATOM	214	C	VAL A 643	45.440	29.441	22.099	1.00	29.51	A	ATOM	265	CA	MET A 650	37.943	24.047	19.346	1.00	34.92	A
ATOM	215	O	VAL A 643	44.449	28.699	22.071	1.00	26.58	A	ATOM	266	CB	MET A 650	39.267	23.405	19.772	1.00	33.03	A
ATOM	216	N	GLN A 644	45.383	30.718	22.471	1.00	30.11	A	ATOM	267	CG	MET A 650	39.795	23.910	21.109	1.00	29.50	A
ATOM	217	CA	GLN A 644	44.133	31.351	22.879	1.00	33.01	A	ATOM	268	SD	MET A 650	41.556	23.603	21.324	1.00	29.20	A
ATOM	218	CB	GLN A 644	44.367	32.817	23.227	1.00	36.02	A	ATOM	269	CE	MET A 650	41.642	21.805	21.052	1.00	24.15	A
ATOM	219	CG	GLN A 644	45.048	33.057	24.558	1.00	41.78	A	ATOM	270	C	MET A 650	36.903	23.858	20.438	1.00	34.75	A
ATOM	220	CD	GLN A 644	45.132	34.536	24.884	1.00	45.52	A	ATOM	271	O	MET A 650	36.658	24.757	21.237	1.00	33.78	A
ATOM	221	OE1	GLN A 644	44.194	35.292	24.616	1.00	49.19	A	ATOM	272	N	ASN A 651	36.294	22.680	20.468	1.00	35.64	A
ATOM	222	NE2	GLN A 644	46.248	34.957	25.472	1.00	47.22	A	ATOM	273	CA	ASN A 651	35.283	22.387	21.471	1.00	37.36	A
ATOM	223	C	GLN A 644	43.067	31.274	21.795	1.00	33.32	A	ATOM	274	CB	ASN A 651	34.296	21.350	20.940	1.00	39.99	A
ATOM	224	O	GLN A 644	41.885	31.082	22.085	1.00	33.13	A	ATOM	275	CG	ASN A 651	33.320	21.943	19.952	1.00	43.45	A
ATOM	225	N	GLN A 645	43.488	31.439	20.548	1.00	31.47	A	ATOM	276	OD1	ASN A 651	32.568	22.862	20.289	1.00	44.07	A
ATOM	226	CA	GLN A 645	42.571	31.387	19.424	1.00	31.69	A	ATOM	277	ND2	ASN A 651	33.324	21.427	18.724	1.00	43.60	A
ATOM	227	CB	GLN A 645	43.287	31.857	18.150	1.00	33.25	A	ATOM	278	C	ASN A 651	35.888	21.891	22.766	1.00	35.69	A
ATOM	228	CG	GLN A 645	42.539	31.601	16.842	1.00	38.06	A	ATOM	279	O	ASN A 651	35.348	22.136	23.843	1.00	35.39	A
ATOM	229	CD	GLN A 645	41.087	32.044	16.898	1.00	41.72	A	ATOM	280	N	ASP A 652	37.018	21.205	22.662	1.00	34.83	A
ATOM	230	OE1	GLN A 645	40.750	33.036	17.552	1.00	44.39	A	ATOM	281	CA	ASP A 652	37.673	20.657	23.840	1.00	35.28	A
ATOM	231	NE2	GLN A 645	40.220	31.316	16.199	1.00	42.69	A	ATOM	282	CB	ASP A 652	37.529	19.129	23.834	1.00	37.20	A
ATOM	232	C	GLN A 645	42.051	29.967	19.251	1.00	30.46	A	ATOM	283	CG	ASP A 652	37.974	18.491	25.136	1.00	37.98	A
ATOM	233	O	GLN A 645	40.842	29.743	19.169	1.00	28.81	A	ATOM	284	OD1	ASP A 652	38.710	19.141	25.909	1.00	37.06	A
ATOM	234	N	AIA A 646	42.975	29.013	19.202	1.00	31.16	A	ATOM	285	OD2	ASP A 652	37.593	17.326	25.380	1.00	41.75	A
ATOM	235	CA	AIA A 646	42.626	27.604	19.036	1.00	31.25	A	ATOM	286	C	ASP A 652	39.151	21.036	23.878	1.00	33.58	A
ATOM	236	CB	AIA A 646	43.891	26.747	19.105	1.00	30.19	A	ATOM	287	O	ASP A 652	39.934	20.573	23.052	1.00	34.09	A
ATOM	237	C	AIA A 646	41.621	27.151	20.096	1.00	31.57	A	ATOM	288	N	PRO A 653	39.553	21.879	24.844	1.00	33.25	A
ATOM	238	O	AIA A 646	40.625	26.504	19.780	1.00	32.23	A	ATOM	289	CD	PRO A 653	38.749	22.522	25.900	1.00	32.88	A
ATOM	239	N	VAL A 647	41.886	27.508	21.349	1.00	32.85	A	ATOM	290	CA	PRO A 653	40.964	22.278	24.935	1.00	32.22	A
ATOM	240	CA	VAL A 647	41.025	27.138	22.470	1.00	35.18	A	ATOM	291	CB	PRO A 653	40.955	23.346	26.028	1.00	31.91	A
ATOM	241	CB	VAL A 647	41.628	27.647	23.804	1.00	35.10	A	ATOM	292	CG	PRO A 653	39.804	22.932	26.901	1.00	33.73	A
ATOM	242	CG1	VAL A 647	40.617	27.524	24.926	1.00	35.24	A	ATOM	293	C	PRO A 653	41.890	21.104	25.264	1.00	31.41	A
ATOM	243	CG2	VAL A 647	42.877	26.838	24.140	1.00	34.83	A	ATOM	294	O	PRO A 653	43.110	21.227	25.180	1.00	29.79	A
ATOM	244	C	VAL A 647	39.584	27.640	22.327	1.00	37.67	A	ATOM	295	N	SER A 654	41.295	19.966	25.624	1.00	31.41	A
ATOM	245	O	VAL A 647	38.636	26.954	22.725	1.00	36.88	A	ATOM	296	CA	SER A 654	42.048	18.758	25.969	1.00	31.00	A
ATOM	246	N	LYS A 648	39.413	28.833	21.763	1.00	38.99	A	ATOM	297	CB	SER A 654	41.377	18.041	27.139	1.00	31.32	A
ATOM	247	CA	LYS A 648	38.075	29.373	21.564	1.00	39.75	A	ATOM	298	OG	SER A 654	41.154	18.932	28.217	1.00	34.09	A
ATOM	248	CB	LYS A 648	38.137	30.873	21.259	1.00	41.77	A	ATOM	299	C	SER A 654	42.139	17.796	24.787	1.00	31.09	A
ATOM	249	CG	LYS A 648	38.199	31.749	22.500	1.00	46.15	A	ATOM	300	O	SER A 654	42.518	16.634	24.951	1.00	29.80	A
ATOM	250	CD	LYS A 648	39.462	31.492	23.314	1.00	49.32	A	ATOM	301	N	GLN A 655	41.780	18.283	23.603	1.00	29.73	A
ATOM	251	CE	LYS A 648	39.382	32.157	24.680	1.00	51.64	A	ATOM	302	CA	GLN A 655	41.815	17.480	22.381	1.00	29.19	A
ATOM	252	NZ	LYS A 648	38.982	33.590	24.570	1.00	54.34	A	ATOM	303	CB	GLN A 655	41.397	18.347	21.189	1.00	30.04	A

ATOM	304	CG	GLN A 655	41.272	17.600	19.871	1.00	32.33	A	ATOM	355	N	THR A 662	48.378	16.235	24.143	1.00	24.53	A
ATOM	305	CD	GLN A 655	40.221	16.512	19.927	1.00	33.17	A	ATOM	356	CA	THR A 662	47.506	17.401	24.225	1.00	21.49	A
ATOM	306	OE1	GLN A 655	39.067	16.770	20.269	1.00	31.58	A	ATOM	357	CB	THR A 662	46.604	17.343	25.482	1.00	22.99	A
ATOM	307	NE2	GLN A 655	40.615	15.286	19.593	1.00	34.79	A	ATOM	358	OG1	THR A 662	47.418	17.396	26.665	1.00	25.61	A
ATOM	308	C	GLN A 655	43.206	16.898	22.120	1.00	28.57	A	ATOM	359	CG2	THR A 662	45.785	16.063	25.490	1.00	21.13	A
ATOM	309	O	GLN A 655	44.167	17.642	21.931	1.00	29.36	A	ATOM	360	C	THR A 662	48.336	18.677	24.287	1.00	19.80	A
ATOM	310	N	PRO A 656	43.332	15.556	22.112	1.00	28.07	A	ATOM	361	O	THR A 662	49.542	18.635	24.529	1.00	20.50	A
ATOM	311	CD	PRO A 656	42.362	14.579	22.644	1.00	26.92	A	ATOM	362	N	ALA A 663	47.685	19.812	24.075	1.00	18.98	A
ATOM	312	CA	PRO A 656	44.625	14.906	21.866	1.00	28.25	A	ATOM	363	CA	ALA A 663	48.364	21.100	24.118	1.00	19.37	A
ATOM	313	CB	PRO A 656	44.550	13.674	22.748	1.00	26.47	A	ATOM	364	CB	ALA A 663	47.348	22.232	23.924	1.00	19.41	A
ATOM	314	CG	PRO A 656	43.129	13.256	22.546	1.00	26.21	A	ATOM	365	C	ALA A 663	49.096	21.286	25.446	1.00	19.15	A
ATOM	315	C	PRO A 656	44.809	14.514	20.400	1.00	29.40	A	ATOM	366	O	ALA A 663	50.223	21.774	25.474	1.00	18.54	A
ATOM	316	O	PRO A 656	43.836	14.385	19.662	1.00	30.23	A	ATOM	367	N	LEU A 664	48.448	20.898	26.544	1.00	19.98	A
ATOM	317	N	ASN A 657	46.057	14.332	19.980	1.00	30.19	A	ATOM	368	CA	LEU A 664	49.046	21.035	27.866	1.00	20.65	A
ATOM	318	CA	ASN A 657	46.316	13.901	18.619	1.00	31.90	A	ATOM	369	CB	LEU A 664	48.062	20.592	28.954	1.00	20.18	A
ATOM	319	CB	ASN A 657	47.666	14.432	18.105	1.00	30.70	A	ATOM	370	CG	LEU A 664	48.549	20.718	30.408	1.00	22.87	A
ATOM	320	CG	ASN A 657	48.865	13.884	18.870	1.00	31.75	A	ATOM	371	CD1	LEU A 664	48.957	22.158	30.699	1.00	19.46	A
ATOM	321	OD1	ASN A 657	48.751	12.960	19.673	1.00	31.10	A	ATOM	372	CD2	LEU A 664	47.442	20.275	31.366	1.00	21.05	A
ATOM	322	ND2	ASN A 657	50.034	14.453	18.603	1.00	30.18	A	ATOM	373	C	LEU A 664	50.342	20.237	27.980	1.00	22.54	A
ATOM	323	C	ASN A 657	46.297	12.368	18.643	1.00	34.53	A	ATOM	374	O	LEU A 664	51.305	20.703	28.586	1.00	20.96	A
ATOM	324	O	ASN A 657	45.835	11.764	19.618	1.00	35.13	A	ATOM	375	N	HIS A 665	50.370	19.038	27.402	1.00	23.35	A
ATOM	325	N	GLU A 658	46.791	11.739	17.583	1.00	36.50	A	ATOM	376	CA	HIS A 665	51.580	18.226	27.449	1.00	23.89	A
ATOM	326	CA	GLU A 658	46.802	10.282	17.504	1.00	39.76	A	ATOM	377	CB	HIS A 665	51.423	16.931	26.641	1.00	24.89	A
ATOM	327	CB	GLU A 658	47.240	9.836	16.107	1.00	43.45	A	ATOM	378	CG	HIS A 665	50.657	15.853	27.343	1.00	24.90	A
ATOM	328	CG	GLU A 658	46.101	9.336	15.231	1.00	49.71	A	ATOM	379	CD2	HIS A 665	51.077	14.827	28.121	1.00	22.97	A
ATOM	329	CD	GLU A 658	46.284	7.882	14.812	1.00	54.58	A	ATOM	380	ND1	HIS A 665	49.288	15.725	27.244	1.00	26.82	A
ATOM	330	OE1	GLU A 658	47.266	7.592	14.091	1.00	57.23	A	ATOM	381	CE1	HIS A 665	48.898	14.663	27.927	1.00	25.66	A
ATOM	331	OE2	GLU A 658	45.454	7.029	15.207	1.00	55.81	A	ATOM	382	NE2	HIS A 665	49.964	14.101	28.469	1.00	25.24	A
ATOM	332	C	GLU A 658	47.697	9.628	18.548	1.00	39.60	A	ATOM	383	C	HIS A 665	52.746	19.011	26.858	1.00	24.01	A
ATOM	333	O	GLU A 658	47.560	8.439	18.830	1.00	40.38	A	ATOM	384	O	HIS A 665	53.815	19.095	27.455	1.00	24.41	A
ATOM	334	N	GLU A 659	48.609	10.405	19.119	1.00	38.44	A	ATOM	385	N	ASN A 666	52.524	19.584	25.679	1.00	23.97	A
ATOM	335	CA	GLU A 659	49.530	9.896	20.125	1.00	38.16	A	ATOM	386	CA	ASN A 666	53.551	20.350	24.974	1.00	25.51	A
ATOM	336	CB	GLU A 659	50.926	10.464	19.871	1.00	43.38	A	ATOM	387	CB	ASN A 666	53.095	20.629	23.541	1.00	24.68	A
ATOM	337	CG	GLU A 659	51.541	10.021	18.551	1.00	49.71	A	ATOM	388	CG	ASN A 666	53.376	19.474	22.610	1.00	25.74	A
ATOM	338	CD	GLU A 659	52.078	8.598	18.603	1.00	54.84	A	ATOM	389	OD1	ASN A 666	53.648	18.355	23.053	1.00	23.88	A
ATOM	339	OE1	GLU A 659	51.300	7.670	18.930	1.00	56.44	A	ATOM	390	ND2	ASN A 666	53.305	19.734	21.306	1.00	23.97	A
ATOM	340	OE2	GLU A 659	53.283	8.410	18.316	1.00	57.46	A	ATOM	391	C	ASN A 666	53.950	21.661	25.642	1.00	24.94	A
ATOM	341	C	GLU A 659	49.093	10.229	21.550	1.00	35.63	A	ATOM	392	O	ASN A 666	55.113	22.050	25.596	1.00	25.53	A
ATOM	342	O	GLU A 659	49.833	10.001	22.502	1.00	36.16	A	ATOM	393	N	ALA A 667	52.987	22.344	26.248	1.00	25.83	A
ATOM	343	N	GLY A 660	47.892	10.771	21.693	1.00	33.22	A	ATOM	394	CA	ALA A 667	53.271	23.605	26.918	1.00	27.57	A
ATOM	344	CA	GLY A 660	47.395	11.119	23.011	1.00	31.31	A	ATOM	395	CB	ALA A 667	51.983	24.222	27.442	1.00	26.86	A
ATOM	345	C	GLY A 660	48.003	12.402	23.544	1.00	30.13	A	ATOM	396	C	ALA A 667	54.230	23.334	28.071	1.00	28.50	A
ATOM	346	O	GLY A 660	47.758	12.782	24.690	1.00	29.18	A	ATOM	397	O	ALA A 667	55.135	24.126	28.344	1.00	28.26	A
ATOM	347	N	ILE A 661	48.793	13.072	22.711	1.00	27.98	A	ATOM	398	N	ILE A 668	54.018	22.202	28.736	1.00	28.64	A
ATOM	348	CA	ILE A 661	49.438	14.315	23.107	1.00	27.74	A	ATOM	399	CA	ILE A 668	54.842	21.785	29.860	1.00	29.35	A
ATOM	349	CB	ILE A 661	50.661	14.606	22.216	1.00	28.93	A	ATOM	400	CB	ILE A 668	54.273	20.501	30.505	1.00	28.30	A
ATOM	350	CG2	ILE A 661	51.304	15.940	22.613	1.00	29.51	A	ATOM	401	CG2	ILE A 668	55.267	19.934	31.513	1.00	27.87	A
ATOM	351	CG1	ILE A 661	51.681	13.480	22.368	1.00	29.78	A	ATOM	402	CG1	ILE A 668	52.923	20.812	31.158	1.00	27.53	A
ATOM	352	CD1	ILE A 661	52.806	13.541	21.360	1.00	34.10	A	ATOM	403	CD1	ILE A 668	52.223	19.605	31.763	1.00	26.60	A
ATOM	353	C	ILE A 661	48.478	15.499	23.037	1.00	26.80	A	ATOM	404	C	ILE A 668	56.275	21.526	29.412	1.00	30.72	A
ATOM	354	O	ILE A 661	47.838	15.733	22.010	1.00	27.22	A	ATOM	405	O	ILE A 668	57.224	22.086	29.964	1.00	30.11	A

ATOM	406	N	CYS A 669	56.427	20.673	28.408	1.00	34.07	A	ATOM	457	CB	VAL A 676	51.323	25.780	31.875	1.00	30.79	A
ATOM	407	CA	CYS A 669	57.744	20.342	27.888	1.00	38.64	A	ATOM	458	CG1	VAL A 676	50.761	24.853	32.956	1.00	29.43	A
ATOM	408	CB	CYS A 669	57.612	19.262	26.805	1.00	42.11	A	ATOM	459	CG2	VAL A 676	52.345	25.030	31.027	1.00	27.20	A
ATOM	409	SG	CYS A 669	57.049	17.652	27.474	1.00	50.37	A	ATOM	460	C	VAL A 676	49.056	26.885	31.850	1.00	30.26	A
ATOM	410	C	CYS A 669	58.466	21.581	27.345	1.00	39.01	A	ATOM	461	O	VAL A 676	47.994	26.280	31.974	1.00	29.05	A
ATOM	411	C	CYS A 669	59.695	21.648	27.362	1.00	39.04	A	ATOM	462	N	ASP A 677	49.294	28.046	32.453	1.00	31.11	A
ATOM	412	N	GLY A 670	57.701	22.565	26.882	1.00	38.69	A	ATOM	463	CA	ASP A 677	48.283	28.661	33.309	1.00	34.47	A
ATOM	413	CA	GLY A 670	58.304	23.777	26.366	1.00	38.10	A	ATOM	464	CB	ASP A 677	48.795	29.973	33.899	1.00	38.00	A
ATOM	414	C	GLY A 670	58.604	24.797	27.452	1.00	38.92	A	ATOM	465	CG	ASP A 677	50.041	29.784	34.739	1.00	44.44	A
ATOM	415	O	GLY A 670	59.183	25.851	27.179	1.00	39.31	A	ATOM	466	OD1	ASP A 677	50.086	28.804	35.521	1.00	47.41	A
ATOM	416	N	ALA A 671	58.215	24.492	28.686	1.00	37.19	A	ATOM	467	OD2	ASP A 677	50.974	30.613	34.623	1.00	49.00	A
ATOM	417	CA	ALA A 671	58.438	25.404	29.803	1.00	37.27	A	ATOM	468	C	ASP A 677	47.006	28.919	32.529	1.00	34.22	A
ATOM	418	CB	ALA A 671	59.930	25.630	30.008	1.00	36.44	A	ATOM	469	O	ASP A 677	45.903	28.656	33.018	1.00	34.62	A
ATOM	419	C	ALA A 671	57.741	26.735	29.540	1.00	37.05	A	ATOM	470	N	PHE A 678	47.156	29.430	31.311	1.00	31.61	A
ATOM	420	O	ALA A 671	58.260	27.795	28.874	1.00	37.81	A	ATOM	471	CA	PHE A 678	46.006	29.703	30.473	1.00	29.95	A
ATOM	421	N	ASN A 672	56.556	26.673	28.947	1.00	37.16	A	ATOM	472	CB	PHE A 678	46.448	30.233	29.107	1.00	30.59	A
ATOM	422	CA	ASN A 672	55.800	27.878	28.628	1.00	37.82	A	ATOM	473	CG	PHE A 678	45.322	30.349	28.123	1.00	29.67	A
ATOM	423	CB	ASN A 672	55.188	27.728	27.233	1.00	36.96	A	ATOM	474	CD1	PHE A 678	44.319	31.295	28.305	1.00	30.67	A
ATOM	424	CG	ASN A 672	56.256	27.558	26.160	1.00	37.30	A	ATOM	475	CD2	PHE A 678	45.220	29.456	27.056	1.00	31.03	A
ATOM	425	OD1	ASN A 672	57.068	28.458	25.939	1.00	35.39	A	ATOM	476	CE1	PHE A 678	43.223	31.346	27.443	1.00	33.20	A
ATOM	426	ND2	ASN A 672	56.274	26.397	25.507	1.00	36.97	A	ATOM	477	CE2	PHE A 678	44.131	29.496	26.188	1.00	30.54	A
ATOM	427	C	ASN A 672	54.742	28.128	29.691	1.00	37.80	A	ATOM	478	CZ	PHE A 678	43.129	30.440	26.381	1.00	33.29	A
ATOM	428	O	ASN A 672	53.542	27.939	29.475	1.00	37.14	A	ATOM	479	C	PHE A 678	45.171	28.444	30.269	1.00	30.28	A
ATOM	429	N	TYR A 673	55.227	28.564	30.849	1.00	37.14	A	ATOM	480	O	PHE A 678	43.963	28.455	30.486	1.00	31.53	A
ATOM	430	CA	TYR A 673	54.405	28.835	32.017	1.00	36.18	A	ATOM	481	N	LEU A 679	45.824	27.363	29.846	1.00	31.15	A
ATOM	431	CB	TYR A 673	55.251	29.576	33.050	1.00	35.71	A	ATOM	482	CA	LEU A 679	45.150	26.092	29.591	1.00	30.55	A
ATOM	432	CG	TYR A 673	56.515	28.823	33.396	1.00	35.55	A	ATOM	483	CB	LEU A 679	46.135	25.091	28.981	1.00	29.02	A
ATOM	433	CD1	TYR A 673	56.469	27.693	34.212	1.00	34.06	A	ATOM	484	CG	LEU A 679	46.708	25.470	27.603	1.00	29.17	A
ATOM	434	CE1	TYR A 673	57.613	26.945	34.481	1.00	34.40	A	ATOM	485	CD1	LEU A 679	47.907	24.586	27.270	1.00	28.10	A
ATOM	435	CD2	TYR A 673	57.752	29.198	32.854	1.00	35.02	A	ATOM	486	CD2	LEU A 679	45.627	25.336	26.541	1.00	26.06	A
ATOM	436	CE2	TYR A 673	58.908	28.455	33.113	1.00	35.10	A	ATOM	487	C	LEU A 679	44.502	25.492	30.832	1.00	31.15	A
ATOM	437	CZ	TYR A 673	58.825	27.324	33.929	1.00	35.91	A	ATOM	488	O	LEU A 679	43.404	24.940	30.757	1.00	32.97	A
ATOM	438	OH	TYR A 673	59.943	26.555	34.174	1.00	37.96	A	ATOM	489	N	ILE A 680	45.170	25.588	31.975	1.00	32.28	A
ATOM	439	C	TYR A 673	53.128	29.606	31.729	1.00	35.88	A	ATOM	490	CA	ILE A 680	44.602	25.047	33.207	1.00	32.48	A
ATOM	440	O	TYR A 673	52.069	29.286	32.268	1.00	34.91	A	ATOM	491	CB	ILE A 680	45.592	25.160	34.387	1.00	31.30	A
ATOM	441	N	SER A 674	53.226	30.618	30.876	1.00	35.72	A	ATOM	492	CG2	ILE A 680	44.877	24.862	35.705	1.00	34.20	A
ATOM	442	CA	SER A 674	52.066	31.432	30.546	1.00	36.56	A	ATOM	493	CG1	ILE A 680	46.746	24.178	34.185	1.00	32.48	A
ATOM	443	CB	SER A 674	52.494	32.623	29.688	1.00	38.83	A	ATOM	494	CD1	ILE A 680	47.783	24.205	35.288	1.00	31.45	A
ATOM	444	OG	SER A 674	51.449	33.572	29.592	1.00	43.36	A	ATOM	495	C	ILE A 680	43.323	25.803	33.539	1.00	32.04	A
ATOM	445	C	SER A 674	50.994	30.614	29.819	1.00	35.51	A	ATOM	496	O	ILE A 680	42.279	25.201	33.779	1.00	32.15	A
ATOM	446	O	SER A 674	49.802	30.776	30.076	1.00	35.02	A	ATOM	497	N	THR A 681	43.415	27.127	33.540	1.00	32.54	A
ATOM	447	N	ILE A 675	51.415	29.734	28.914	1.00	33.87	A	ATOM	498	CA	THR A 681	42.272	27.991	33.823	1.00	33.55	A
ATOM	448	CA	ILE A 675	50.454	28.910	28.192	1.00	33.48	A	ATOM	499	CB	THR A 681	42.671	29.478	33.720	1.00	33.01	A
ATOM	449	CB	ILE A 675	51.099	28.180	26.980	1.00	34.64	A	ATOM	500	OG1	THR A 681	43.653	29.778	34.718	1.00	33.84	A
ATOM	450	CG2	ILE A 675	50.175	27.067	26.488	1.00	34.72	A	ATOM	501	CG2	THR A 681	41.462	30.375	33.916	1.00	34.35	A
ATOM	451	CG1	ILE A 675	51.362	29.169	25.837	1.00	36.37	A	ATOM	502	C	THR A 681	41.134	27.726	32.838	1.00	33.18	A
ATOM	452	CD1	ILE A 675	52.572	30.038	26.034	1.00	40.16	A	ATOM	503	O	THR A 681	39.966	27.800	33.201	1.00	34.04	A
ATOM	453	C	ILE A 675	49.841	27.867	29.126	1.00	30.92	A	ATOM	504	N	ALA A 682	41.481	27.417	31.592	1.00	32.83	A
ATOM	454	O	ILE A 675	48.653	27.565	29.031	1.00	29.86	A	ATOM	505	CA	ALA A 682	40.481	27.140	30.565	1.00	33.44	A
ATOM	455	N	VAL A 676	50.652	27.324	30.027	1.00	29.27	A	ATOM	506	CB	ALA A 682	41.117	27.232	29.183	1.00	32.79	A
ATOM	456	CA	VAL A 676	50.170	26.317	30.969	1.00	30.53	A	ATOM	507	C	ALA A 682	39.823	25.764	30.753	1.00	34.32	A

ATOM	508	O	ALA A 682	38.882	25.412	30.039	1.00	34.46	A	ATOM	559	OD2	ASP A 690	52.684	10.842	26.491	1.00	29.78	A
ATOM	509	N	GLY A 683	40.326	24.982	31.706	1.00	34.21	A	ATOM	560	C	ASP A 690	49.184	8.487	25.921	1.00	30.49	A
ATOM	510	CA	GLY A 683	39.744	23.676	31.959	1.00	33.43	A	ATOM	561	O	ASP A 690	48.980	7.670	26.823	1.00	31.28	A
ATOM	511	C	GLY A 683	40.390	22.499	31.245	1.00	33.63	A	ATOM	562	N	SER A 691	49.469	8.121	24.679	1.00	31.74	A
ATOM	512	O	GLY A 683	39.772	21.441	31.107	1.00	33.34	A	ATOM	563	CA	SER A 691	49.533	6.716	24.304	1.00	34.35	A
ATOM	513	N	ALA A 684	41.626	22.673	30.787	1.00	30.78	A	ATOM	564	CB	SER A 691	50.047	6.582	22.865	1.00	35.85	A
ATOM	514	CA	ALA A 684	42.336	21.595	30.115	1.00	28.90	A	ATOM	565	OG	SER A 691	51.333	7.161	22.725	1.00	38.94	A
ATOM	515	CB	ALA A 684	43.701	22.087	29.648	1.00	27.11	A	ATOM	566	C	SER A 691	50.374	5.856	25.251	1.00	34.09	A
ATOM	516	C	ALA A 684	42.500	20.436	31.107	1.00	27.12	A	ATOM	567	O	SER A 691	50.100	4.665	25.406	1.00	35.98	A
ATOM	517	O	ALA A 684	42.897	20.649	32.243	1.00	27.16	A	ATOM	568	N	HIS A 692	51.387	6.448	25.882	1.00	33.00	A
ATOM	518	N	ASN A 685	42.185	19.218	30.673	1.00	25.70	A	ATOM	569	CA	HIS A 692	52.244	5.709	26.816	1.00	31.98	A
ATOM	519	CA	ASN A 685	42.296	18.033	31.521	1.00	24.13	A	ATOM	570	CB	HIS A 692	53.695	6.195	26.735	1.00	34.71	A
ATOM	520	CB	ASN A 685	41.839	16.799	30.736	1.00	23.36	A	ATOM	571	CG	HIS A 692	54.377	5.858	25.444	1.00	37.96	A
ATOM	521	CG	ASN A 685	41.892	15.524	31.556	1.00	23.03	A	ATOM	572	CD2	HIS A 692	53.895	5.359	24.282	1.00	39.22	A
ATOM	522	OD1	ASN A 685	42.394	15.506	32.685	1.00	24.86	A	ATOM	573	ND1	HIS A 692	55.726	6.062	25.242	1.00	39.48	A
ATOM	523	ND2	ASN A 685	41.384	14.443	30.986	1.00	21.40	A	ATOM	574	CE1	HIS A 692	56.044	5.706	24.011	1.00	39.87	A
ATOM	524	C	ASN A 685	43.733	17.842	32.019	1.00	23.73	A	ATOM	575	NE2	HIS A 692	54.950	5.276	23.407	1.00	40.05	A
ATOM	525	O	ASN A 685	44.605	17.386	31.272	1.00	22.13	A	ATOM	576	C	HIS A 692	51.773	5.810	28.268	1.00	30.18	A
ATOM	526	N	VAL A 686	43.972	18.177	33.287	1.00	23.51	A	ATOM	577	O	HIS A 692	52.417	5.290	29.180	1.00	28.80	A
ATOM	527	CA	VAL A 686	45.315	18.062	33.866	1.00	23.44	A	ATOM	578	N	GLY A 693	50.655	6.489	28.486	1.00	28.19	A
ATOM	528	CB	VAL A 686	45.415	18.786	35.249	1.00	23.79	A	ATOM	579	CA	GLY A 693	50.139	6.611	29.835	1.00	26.38	A
ATOM	529	CG1	VAL A 686	45.014	20.253	35.118	1.00	21.57	A	ATOM	580	C	GLY A 693	50.734	7.753	30.629	1.00	25.39	A
ATOM	530	CG2	VAL A 686	44.533	18.086	36.270	1.00	23.36	A	ATOM	581	O	GLY A 693	50.561	7.819	31.845	1.00	25.47	A
ATOM	531	C	VAL A 686	45.797	16.620	34.061	1.00	23.66	A	ATOM	582	N	TRP A 694	51.452	8.644	29.952	1.00	23.41	A
ATOM	532	O	VAL A 686	46.937	16.407	34.476	1.00	24.39	A	ATOM	583	CA	TRP A 694	52.052	9.797	30.616	1.00	22.46	A
ATOM	533	N	ASN A 687	44.945	15.639	33.757	1.00	23.08	A	ATOM	584	CB	TRP A 694	53.239	10.331	29.813	1.00	21.81	A
ATOM	534	CA	ASN A 687	45.303	14.239	33.938	1.00	23.08	A	ATOM	585	CG	TRP A 694	54.493	9.541	29.934	1.00	22.28	A
ATOM	535	CB	ASN A 687	44.357	13.586	34.956	1.00	23.46	A	ATOM	586	CD2	TRP A 694	55.809	10.064	30.142	1.00	23.77	A
ATOM	536	CG	ASN A 687	44.540	14.138	36.349	1.00	21.06	A	ATOM	587	CE2	TRP A 694	56.706	8.971	30.085	1.00	22.78	A
ATOM	537	OD1	ASN A 687	43.583	14.557	36.994	1.00	24.96	A	ATOM	588	CE3	TRP A 694	56.321	11.354	30.365	1.00	23.19	A
ATOM	538	ND2	ASN A 687	45.770	14.135	36.822	1.00	21.73	A	ATOM	589	CD1	TRP A 694	54.637	8.186	29.776	1.00	22.05	A
ATOM	539	C	ASN A 687	45.290	13.383	32.666	1.00	23.68	A	ATOM	590	NE1	TRP A 694	55.963	7.841	29.862	1.00	22.26	A
ATOM	540	O	ASN A 687	45.526	12.174	32.720	1.00	23.15	A	ATOM	591	CZ2	TRP A 694	58.090	9.127	30.242	1.00	23.64	A
ATOM	541	N	SER A 688	45.022	14.005	31.526	1.00	24.03	A	ATOM	592	CZ3	TRP A 694	57.696	11.511	30.520	1.00	23.82	A
ATOM	542	CA	SER A 688	44.960	13.260	30.273	1.00	25.56	A	ATOM	593	CH2	TRP A 694	58.565	10.400	30.457	1.00	26.74	A
ATOM	543	CB	SER A 688	44.583	14.197	29.123	1.00	26.44	A	ATOM	594	C	TRP A 694	51.026	10.916	30.756	1.00	22.10	A
ATOM	544	OG	SER A 688	45.265	15.432	29.246	1.00	34.30	A	ATOM	595	O	TRP A 694	50.418	11.342	29.763	1.00	21.88	A
ATOM	545	C	SER A 688	46.255	12.523	29.955	1.00	25.20	A	ATOM	596	N	THR A 695	50.835	11.389	31.985	1.00	20.37	A
ATOM	546	O	SER A 688	47.315	13.132	29.816	1.00	24.90	A	ATOM	597	CA	THR A 695	49.905	12.480	32.244	1.00	19.73	A
ATOM	547	N	PRO A 689	46.181	11.187	29.845	1.00	24.97	A	ATOM	598	CB	THR A 695	49.226	12.332	33.624	1.00	22.19	A
ATOM	548	CD	PRO A 689	45.056	10.336	30.278	1.00	26.29	A	ATOM	599	OG1	THR A 695	50.210	12.497	34.657	1.00	22.11	A
ATOM	549	CA	PRO A 689	47.362	10.374	29.542	1.00	25.51	A	ATOM	600	CG2	THR A 695	48.572	10.953	33.763	1.00	19.98	A
ATOM	550	CB	PRO A 689	47.047	9.053	30.233	1.00	25.02	A	ATOM	601	C	THR A 695	50.735	13.766	32.262	1.00	19.62	A
ATOM	551	CG	PRO A 689	45.579	8.926	30.013	1.00	28.05	A	ATOM	602	O	THR A 695	51.961	13.719	32.181	1.00	21.10	A
ATOM	552	C	PRO A 689	47.574	10.197	28.047	1.00	24.50	A	ATOM	603	N	PRO A 696	50.078	14.933	32.327	1.00	18.93	A
ATOM	553	O	PRO A 689	46.607	10.078	27.299	1.00	25.02	A	ATOM	604	CD	PRO A 696	48.668	15.190	31.976	1.00	18.34	A
ATOM	554	N	ASP A 690	48.832	10.194	27.611	1.00	25.07	A	ATOM	605	CA	PRO A 696	50.838	16.185	32.363	1.00	18.31	A
ATOM	555	CA	ASP A 690	49.116	9.990	26.199	1.00	27.78	A	ATOM	606	CB	PRO A 696	49.743	17.237	32.449	1.00	17.61	A
ATOM	556	CB	ASP A 690	50.426	10.680	25.782	1.00	28.01	A	ATOM	607	CG	PRO A 696	48.697	16.652	31.538	1.00	20.23	A
ATOM	557	CG	ASP A 690	51.654	10.136	26.498	1.00	28.95	A	ATOM	608	C	PRO A 696	51.809	16.236	33.551	1.00	17.16	A
ATOM	558	OD1	ASP A 690	51.617	9.015	27.048	1.00	29.26	A	ATOM	609	O	PRO A 696	52.894	16.806	33.447	1.00	18.66	A

ATOM	610	N	LEU A 697	51.430	15.622	34.671	1.00	17.41	A	ATOM	661	ND2	ASN A 704	65.464	17.126	36.819	1.00	18.56	A
ATOM	611	CA	LEU A 697	52.295	15.615	35.846	1.00	16.21	A	ATOM	662	C	ASN A 704	61.466	19.874	37.222	1.00	26.84	A
ATOM	612	CB	LEU A 697	51.574	15.022	37.068	1.00	16.88	A	ATOM	663	O	ASN A 704	62.004	20.646	38.019	1.00	26.41	A
ATOM	613	CG	LEU A 697	52.308	15.226	38.404	1.00	18.04	A	ATOM	664	N	ASP A 705	60.377	20.200	36.530	1.00	26.70	A
ATOM	614	CD1	LEU A 697	52.399	16.719	38.713	1.00	16.60	A	ATOM	665	CA	ASP A 705	59.776	21.522	36.670	1.00	27.17	A
ATOM	615	CD2	LEU A 697	51.588	14.496	39.529	1.00	18.83	A	ATOM	666	CB	ASP A 705	59.050	21.920	35.384	1.00	26.24	A
ATOM	616	C	LEU A 697	53.560	14.820	35.562	1.00	16.80	A	ATOM	667	CG	ASP A 705	58.800	23.411	35.310	1.00	30.35	A
ATOM	617	O	LEU A 697	54.647	15.213	35.997	1.00	15.41	A	ATOM	668	OD1	ASP A 705	58.279	23.971	36.299	1.00	31.12	A
ATOM	618	N	HIS A 698	53.423	13.701	34.841	1.00	16.45	A	ATOM	669	OD2	ASP A 705	59.125	24.027	34.272	1.00	32.39	A
ATOM	619	CA	HIS A 698	54.584	12.877	34.495	1.00	16.84	A	ATOM	670	C	ASP A 705	58.813	21.607	37.861	1.00	25.76	A
ATOM	620	CB	HIS A 698	54.171	11.633	33.699	1.00	16.78	A	ATOM	671	O	ASP A 705	57.607	21.399	37.722	1.00	24.90	A
ATOM	621	CG	HIS A 698	53.523	10.562	34.521	1.00	15.26	A	ATOM	672	N	THR A 706	59.368	21.933	39.027	1.00	24.40	A
ATOM	622	CD2	HIS A 698	54.013	9.772	35.506	1.00	15.94	A	ATOM	673	CA	THR A 706	58.606	22.030	40.260	1.00	22.07	A
ATOM	623	ND1	HIS A 698	52.214	10.169	34.330	1.00	15.47	A	ATOM	674	CB	THR A 706	59.532	22.425	41.431	1.00	21.27	A
ATOM	624	CE1	HIS A 698	51.928	9.183	35.163	1.00	18.07	A	ATOM	675	OG1	THR A 706	60.641	21.519	41.479	1.00	21.68	A
ATOM	625	NE2	HIS A 698	53.003	8.922	35.887	1.00	15.82	A	ATOM	676	CG2	THR A 706	58.782	22.363	42.762	1.00	19.77	A
ATOM	626	C	HIS A 698	55.584	13.668	33.648	1.00	16.53	A	ATOM	677	C	THR A 706	57.430	23.003	40.180	1.00	21.36	A
ATOM	627	O	HIS A 698	56.786	13.548	33.835	1.00	16.38	A	ATOM	678	O	THR A 706	56.336	22.689	40.638	1.00	21.69	A
ATOM	628	N	CYS A 699	55.083	14.459	32.703	1.00	18.57	A	ATOM	679	N	VAL A 707	57.652	24.173	39.592	1.00	22.18	A
ATOM	629	CA	CYS A 699	55.953	15.258	31.841	1.00	21.59	A	ATOM	680	CA	VAL A 707	56.600	25.182	39.456	1.00	23.11	A
ATOM	630	CB	CYS A 699	55.144	15.893	30.709	1.00	24.78	A	ATOM	681	CB	VAL A 707	57.160	26.462	38.760	1.00	24.56	A
ATOM	631	SG	CYS A 699	54.209	14.718	29.728	1.00	34.64	A	ATOM	682	CG1	VAL A 707	56.036	27.430	38.419	1.00	23.35	A
ATOM	632	C	CYS A 699	56.654	16.364	32.633	1.00	18.92	A	ATOM	683	CG2	VAL A 707	58.165	27.142	39.686	1.00	24.61	A
ATOM	633	O	CYS A 699	57.831	16.626	32.432	1.00	20.23	A	ATOM	684	C	VAL A 707	55.385	24.639	38.691	1.00	23.71	A
ATOM	634	N	ALA A 700	55.915	17.012	33.527	1.00	20.33	A	ATOM	685	O	VAL A 707	54.241	24.853	39.095	1.00	23.30	A
ATOM	635	CA	ALA A 700	56.470	18.086	34.353	1.00	20.40	A	ATOM	686	N	ILE A 708	55.624	23.931	37.593	1.00	23.74	A
ATOM	636	CB	ALA A 700	55.362	18.711	35.237	1.00	19.78	A	ATOM	687	CA	ILE A 708	54.510	23.360	36.844	1.00	25.35	A
ATOM	637	C	ALA A 700	57.600	17.557	35.228	1.00	18.88	A	ATOM	688	CB	ILE A 708	54.963	22.745	35.508	1.00	25.85	A
ATOM	638	O	ALA A 700	58.629	18.209	35.377	1.00	16.13	A	ATOM	689	CG2	ILE A 708	53.755	22.184	34.772	1.00	25.06	A
ATOM	639	N	ALA A 701	57.397	16.370	35.801	1.00	19.96	A	ATOM	690	CG1	ILE A 708	55.672	23.801	34.655	1.00	29.87	A
ATOM	640	CA	ALA A 701	58.395	15.744	36.664	1.00	20.83	A	ATOM	691	CD1	ILE A 708	54.799	24.972	34.271	1.00	31.87	A
ATOM	641	CB	ALA A 701	57.804	14.515	37.364	1.00	17.17	A	ATOM	692	C	ILE A 708	53.846	22.257	37.681	1.00	24.69	A
ATOM	642	C	ALA A 701	59.646	15.342	35.888	1.00	21.50	A	ATOM	693	O	ILE A 708	52.626	22.206	37.788	1.00	24.47	A
ATOM	643	O	ALA A 701	60.765	15.543	36.371	1.00	21.92	A	ATOM	694	N	CYS A 709	54.653	21.376	38.267	1.00	23.68	A
ATOM	644	N	SER A 702	59.465	14.784	34.689	1.00	21.75	A	ATOM	695	CA	CYS A 709	54.122	20.299	39.101	1.00	24.69	A
ATOM	645	CA	SER A 702	60.615	14.360	33.885	1.00	22.86	A	ATOM	696	CB	CYS A 709	55.257	19.567	39.825	1.00	21.64	A
ATOM	646	CB	SER A 702	60.165	13.590	32.637	1.00	22.10	A	ATOM	697	SG	CYS A 709	56.201	18.454	38.771	1.00	26.87	A
ATOM	647	OG	SER A 702	59.694	14.467	31.632	1.00	26.29	A	ATOM	698	C	CYS A 709	53.142	20.865	40.125	1.00	24.39	A
ATOM	648	C	SER A 702	61.487	15.553	33.479	1.00	23.63	A	ATOM	699	O	CYS A 709	52.037	20.346	40.301	1.00	24.50	A
ATOM	649	O	SER A 702	62.639	15.379	33.111	1.00	24.23	A	ATOM	700	N	MET A 710	53.552	21.938	40.791	1.00	24.76	A
ATOM	650	N	CYS A 703	60.927	16.758	33.542	1.00	25.74	A	ATOM	701	CA	MET A 710	52.708	22.576	41.788	1.00	25.29	A
ATOM	651	CA	CYS A 703	61.662	17.983	33.228	1.00	27.47	A	ATOM	702	CB	MET A 710	53.437	23.765	42.412	1.00	30.52	A
ATOM	652	CB	CYS A 703	60.779	18.976	32.452	1.00	30.02	A	ATOM	703	CG	MET A 710	54.671	23.403	43.212	1.00	37.55	A
ATOM	653	SG	CYS A 703	60.144	18.427	30.852	1.00	42.94	A	ATOM	704	SD	MET A 710	55.397	24.895	43.932	1.00	48.72	A
ATOM	654	C	CYS A 703	62.062	18.635	34.559	1.00	26.41	A	ATOM	705	CE	MET A 710	53.975	25.496	44.914	1.00	42.39	A
ATOM	655	O	CYS A 703	62.634	19.722	34.586	1.00	26.35	A	ATOM	706	C	MET A 710	51.392	23.053	41.185	1.00	21.49	A
ATOM	656	N	ASN A 704	61.741	17.965	35.660	1.00	24.60	A	ATOM	707	O	MET A 710	50.329	22.806	41.741	1.00	21.24	A
ATOM	657	CA	ASN A 704	62.036	18.474	36.997	1.00	25.39	A	ATOM	708	N	ALA A 711	51.475	23.746	40.053	1.00	21.51	A
ATOM	658	CB	ASN A 704	63.546	18.512	37.254	1.00	22.78	A	ATOM	709	CA	ALA A 711	50.285	24.263	39.373	1.00	21.36	A
ATOM	659	CG	ASN A 704	64.187	17.150	37.172	1.00	20.17	A	ATOM	710	CB	ALA A 711	50.684	25.018	38.111	1.00	19.83	A
ATOM	660	OD1	ASN A 704	63.550	16.126	37.441	1.00	22.10	A	ATOM	711	C	ALA A 711	49.331	23.124	39.018	1.00	21.17	A

ATOM	712	O	ALA A 711	48.116	23.273	39.113	1.00	21.72	A	ATOM	763	CB	ILE A 719	49.484	12.335	43.853	1.00	25.02	A
ATOM	713	N	LEU A 712	49.890	21.988	38.610	1.00	21.05	A	ATOM	764	CG2	ILE A 719	50.900	12.305	44.414	1.00	24.81	A
ATOM	714	CA	LEU A 712	49.090	20.824	38.256	1.00	20.48	A	ATOM	765	CG1	ILE A 719	48.829	13.689	44.135	1.00	25.81	A
ATOM	715	CB	LEU A 712	49.956	19.795	37.516	1.00	19.77	A	ATOM	766	CD1	ILE A 719	49.433	14.828	43.342	1.00	24.46	A
ATOM	716	CG	LEU A 712	50.463	20.225	36.131	1.00	18.49	A	ATOM	767	C	ILE A 719	50.047	10.674	42.087	1.00	22.09	A
ATOM	717	CD1	LEU A 712	51.462	19.206	35.595	1.00	16.59	A	ATOM	768	O	ILE A 719	51.226	10.509	41.780	1.00	21.30	A
ATOM	718	CD2	LEU A 712	49.272	20.370	35.175	1.00	19.89	A	ATOM	769	N	PHE A 720	49.195	9.666	42.239	1.00	23.30	A
ATOM	719	C	LEU A 712	48.472	20.195	39.508	1.00	20.43	A	ATOM	770	CA	PHE A 720	49.629	8.281	42.086	1.00	23.33	A
ATOM	720	O	LEU A 712	47.282	19.901	39.539	1.00	21.60	A	ATOM	771	CB	PHE A 720	49.025	7.426	43.205	1.00	22.71	A
ATOM	721	N	VAL A 713	49.285	19.988	40.534	1.00	22.12	A	ATOM	772	CG	PHE A 720	49.674	7.652	44.537	1.00	24.69	A
ATOM	722	CA	VAL A 713	48.803	19.399	41.780	1.00	22.49	A	ATOM	773	CD1	PHE A 720	50.898	7.060	44.833	1.00	25.15	A
ATOM	723	CB	VAL A 713	49.945	19.280	42.818	1.00	22.02	A	ATOM	774	CD2	PHE A 720	49.101	8.516	45.472	1.00	25.91	A
ATOM	724	CG1	VAL A 713	49.382	18.952	44.201	1.00	22.58	A	ATOM	775	CE1	PHE A 720	51.552	7.331	46.042	1.00	26.47	A
ATOM	725	CG2	VAL A 713	50.921	18.212	42.382	1.00	20.70	A	ATOM	776	CE2	PHE A 720	49.745	8.793	46.683	1.00	25.10	A
ATOM	726	C	VAL A 713	47.679	20.246	42.368	1.00	22.46	A	ATOM	777	CZ	PHE A 720	50.974	8.200	46.967	1.00	25.07	A
ATOM	727	O	VAL A 713	46.652	19.722	42.795	1.00	20.44	A	ATOM	778	C	PHE A 720	49.374	7.628	40.741	1.00	23.39	A
ATOM	728	N	GLN A 714	47.875	21.559	42.362	1.00	23.24	A	ATOM	779	O	PHE A 720	49.500	6.410	40.613	1.00	23.95	A
ATOM	729	CA	GLN A 714	46.892	22.487	42.903	1.00	24.45	A	ATOM	780	N	ALA A 721	49.021	8.423	39.737	1.00	23.13	A
ATOM	730	CB	GLN A 714	47.533	23.868	43.089	1.00	27.84	A	ATOM	781	CA	ALA A 721	48.794	7.871	38.410	1.00	23.08	A
ATOM	731	CG	GLN A 714	48.559	23.870	44.226	1.00	32.71	A	ATOM	782	CB	ALA A 721	48.320	8.959	37.453	1.00	22.87	A
ATOM	732	CD	GLN A 714	49.303	25.187	44.374	1.00	37.91	A	ATOM	783	C	ALA A 721	50.121	7.282	37.936	1.00	23.10	A
ATOM	733	OE1	GLN A 714	48.693	26.253	44.488	1.00	40.04	A	ATOM	784	O	ALA A 721	51.192	7.830	38.216	1.00	23.52	A
ATOM	734	NE2	GLN A 714	50.631	25.116	44.382	1.00	39.13	A	ATOM	785	N	THR A 722	50.055	6.154	37.239	1.00	24.19	A
ATOM	735	C	GLN A 714	45.619	22.586	42.080	1.00	24.49	A	ATOM	786	CA	THR A 722	51.262	5.500	36.746	1.00	27.20	A
ATOM	736	O	GLN A 714	44.652	23.223	42.499	1.00	23.79	A	ATOM	787	CB	THR A 722	51.457	4.108	37.379	1.00	27.67	A
ATOM	737	N	HIS A 715	45.605	21.942	40.915	1.00	23.98	A	ATOM	788	OG1	THR A 722	50.311	3.299	37.100	1.00	28.99	A
ATOM	738	CA	HIS A 715	44.417	21.959	40.081	1.00	23.04	A	ATOM	789	CG2	THR A 722	51.644	4.220	38.889	1.00	28.92	A
ATOM	739	CB	HIS A 715	44.653	22.829	38.844	1.00	25.21	A	ATOM	790	C	THR A 722	51.203	5.330	35.241	1.00	26.64	A
ATOM	740	CG	HIS A 715	44.842	24.279	39.168	1.00	28.13	A	ATOM	791	O	THR A 722	50.124	5.179	34.664	1.00	27.71	A
ATOM	741	CD2	HIS A 715	43.954	25.301	39.235	1.00	28.99	A	ATOM	792	N	THR A 723	52.371	5.357	34.612	1.00	28.76	A
ATOM	742	ND1	HIS A 715	46.057	24.804	39.557	1.00	28.65	A	ATOM	793	CA	THR A 723	52.477	5.212	33.162	1.00	31.03	A
ATOM	743	CE1	HIS A 715	45.909	26.084	39.849	1.00	28.27	A	ATOM	794	CB	THR A 723	53.876	5.607	32.674	1.00	30.74	A
ATOM	744	NE2	HIS A 715	44.642	26.410	39.663	1.00	29.48	A	ATOM	795	OG1	THR A 723	54.843	4.757	33.301	1.00	32.52	A
ATOM	745	C	HIS A 715	43.893	20.572	39.694	1.00	23.27	A	ATOM	796	CG2	THR A 723	54.187	7.051	33.025	1.00	29.30	A
ATOM	746	O	HIS A 715	43.311	20.389	38.624	1.00	22.44	A	ATOM	797	C	THR A 723	52.237	3.769	32.731	1.00	32.58	A
ATOM	747	N	GLY A 716	44.113	19.592	40.566	1.00	21.18	A	ATOM	798	O	THR A 723	52.273	2.849	33.555	1.00	29.91	A
ATOM	748	CA	GLY A 716	43.585	18.261	40.321	1.00	20.03	A	ATOM	799	N	LEU A 724	51.985	3.584	31.438	1.00	35.54	A
ATOM	749	C	GLY A 716	44.414	17.165	39.688	1.00	19.83	A	ATOM	800	CA	LEU A 724	51.773	2.252	30.868	1.00	40.32	A
ATOM	750	O	GLY A 716	43.841	16.245	39.113	1.00	22.48	A	ATOM	801	CB	LEU A 724	50.986	2.327	29.556	1.00	39.93	A
ATOM	751	N	ALA A 717	45.737	17.234	39.776	1.00	18.59	A	ATOM	802	CG	LEU A 724	49.467	2.423	29.528	1.00	42.08	A
ATOM	752	CA	ALA A 717	46.574	16.190	39.193	1.00	19.86	A	ATOM	803	CD1	LEU A 724	49.035	2.610	28.086	1.00	44.07	A
ATOM	753	CB	ALA A 717	48.016	16.673	39.068	1.00	18.81	A	ATOM	804	CD2	LEU A 724	48.837	1.165	30.111	1.00	43.26	A
ATOM	754	C	ALA A 717	46.523	14.926	40.053	1.00	20.17	A	ATOM	805	C	LEU A 724	53.142	1.677	30.552	1.00	41.96	A
ATOM	755	O	ALA A 717	46.537	15.006	41.274	1.00	18.98	A	ATOM	806	O	LEU A 724	53.434	0.515	30.837	1.00	42.64	A
ATOM	756	N	ALA A 718	46.459	13.762	39.410	1.00	20.73	A	ATOM	807	N	SER A 725	53.970	2.523	29.948	1.00	44.85	A
ATOM	757	CA	ALA A 718	46.426	12.499	40.139	1.00	20.86	A	ATOM	808	CA	SER A 725	55.321	2.168	29.537	1.00	47.01	A
ATOM	758	CB	ALA A 718	45.774	11.413	39.300	1.00	19.62	A	ATOM	809	CB	SER A 725	56.004	3.399	28.921	1.00	47.70	A
ATOM	759	C	ALA A 718	47.854	12.108	40.504	1.00	22.00	A	ATOM	810	OG	SER A 725	56.074	4.477	29.845	1.00	48.04	A
ATOM	760	O	ALA A 718	48.625	11.607	39.675	1.00	22.94	A	ATOM	811	C	SER A 725	56.217	1.574	30.631	1.00	47.92	A
ATOM	761	N	ILE A 719	48.187	12.352	41.763	1.00	21.91	A	ATOM	812	O	SER A 725	57.276	1.023	30.323	1.00	48.88	A
ATOM	762	CA	ILE A 719	49.501	12.078	42.322	1.00	22.91	A	ATOM	813	N	ASP A 726	55.807	1.681	31.896	1.00	47.77	A

ATOM	814	CA	ASP	A	726	56.613	1.146	32.996	1.00	46.76	A	ATOM	865	CG	LXS	A	733	58.092	9.296	34.904	1.00	31.59	A
ATOM	815	CB	ASP	A	726	58.008	1.802	33.011	1.00	49.27	A	ATOM	866	CD	LXS	A	733	59.122	8.724	33.923	1.00	34.91	A
ATOM	816	CG	ASP	A	726	57.967	3.313	32.779	1.00	52.67	A	ATOM	867	CE	LXS	A	733	58.983	7.212	33.696	1.00	36.88	A
ATOM	817	OD1	ASP	A	726	58.980	3.982	33.080	1.00	54.40	A	ATOM	868	NZ	LXS	A	733	57.749	6.804	32.957	1.00	37.43	A
ATOM	818	OD2	ASP	A	726	56.942	3.836	32.287	1.00	54.05	A	ATOM	869	C	LXS	A	733	60.847	11.408	36.566	1.00	27.64	A
ATOM	819	C	ASP	A	726	55.992	1.241	34.392	1.00	44.50	A	ATOM	870	O	LXS	A	733	61.343	11.878	35.536	1.00	25.07	A
ATOM	820	O	ASP	A	726	56.652	0.947	35.387	1.00	43.73	A	ATOM	871	N	CYS	A	734	61.120	11.900	37.775	1.00	26.36	A
ATOM	821	N	GLY	A	727	54.730	1.652	34.463	1.00	42.69	A	ATOM	872	CA	CYS	A	734	62.060	13.012	37.917	1.00	26.51	A
ATOM	822	CA	GLY	A	727	54.041	1.751	35.742	1.00	38.85	A	ATOM	873	CB	CYS	A	734	62.194	13.406	39.391	1.00	25.87	A
ATOM	823	C	GLY	A	727	54.632	2.694	36.777	1.00	36.52	A	ATOM	874	SG	CYS	A	734	60.696	14.167	40.054	1.00	25.57	A
ATOM	824	O	GLY	A	727	54.474	2.468	37.978	1.00	35.40	A	ATOM	875	C	CYS	A	734	63.414	12.572	37.330	1.00	25.38	A
ATOM	825	N	ALA	A	728	55.293	3.756	36.326	1.00	33.03	A	ATOM	876	O	CYS	A	734	63.860	11.446	37.559	1.00	25.72	A
ATOM	826	CA	ALA	A	728	55.904	4.715	37.240	1.00	31.87	A	ATOM	877	N	ASP	A	735	64.054	13.458	36.569	1.00	25.21	A
ATOM	827	CB	ALA	A	728	57.216	5.224	36.648	1.00	31.31	A	ATOM	878	CA	ASP	A	735	65.323	13.153	35.893	1.00	24.48	A
ATOM	828	C	ALA	A	728	55.000	5.903	37.585	1.00	30.03	A	ATOM	879	CB	ASP	A	735	65.315	13.774	34.489	1.00	25.89	A
ATOM	829	O	ALA	A	728	54.120	6.281	36.809	1.00	30.07	A	ATOM	880	CG	ASP	A	735	66.313	13.112	33.534	1.00	27.87	A
ATOM	830	N	THR	A	729	55.234	6.489	38.756	1.00	27.42	A	ATOM	881	OD1	ASP	A	735	67.184	12.334	33.980	1.00	25.88	A
ATOM	831	CA	THR	A	729	54.471	7.646	39.206	1.00	25.08	A	ATOM	882	OD2	ASP	A	735	66.223	13.384	32.318	1.00	29.94	A
ATOM	832	CB	THR	A	729	54.269	7.620	40.747	1.00	25.56	A	ATOM	883	C	ASP	A	735	66.564	13.636	36.642	1.00	23.26	A
ATOM	833	OG1	THR	A	729	55.541	7.626	41.408	1.00	27.35	A	ATOM	884	O	ASP	A	735	66.857	14.836	36.675	1.00	21.48	A
ATOM	834	CG2	THR	A	729	53.506	6.373	41.168	1.00	26.20	A	ATOM	885	N	PRO	A	736	67.321	12.700	37.238	1.00	22.35	A
ATOM	835	C	THR	A	729	55.253	8.900	38.805	1.00	23.04	A	ATOM	886	CD	PRO	A	736	67.075	11.246	37.266	1.00	24.25	A
ATOM	836	O	THR	A	729	56.386	10.796	38.334	1.00	22.74	A	ATOM	887	CA	PRO	A	736	68.537	13.043	37.987	1.00	23.84	A
ATOM	837	N	ALA	A	730	54.660	8.079	38.975	1.00	22.96	A	ATOM	888	CB	PRO	A	736	68.844	11.752	38.746	1.00	23.34	A
ATOM	838	CA	ALA	A	730	55.352	11.322	38.609	1.00	24.01	A	ATOM	889	CG	PRO	A	736	68.401	10.692	37.774	1.00	25.11	A
ATOM	839	CB	ALA	A	730	54.477	12.532	38.900	1.00	22.52	A	ATOM	890	C	PRO	A	736	69.704	13.518	37.094	1.00	23.43	A
ATOM	840	C	ALA	A	730	56.655	11.432	39.378	1.00	24.91	A	ATOM	891	O	PRO	A	736	70.753	13.914	37.589	1.00	22.65	A
ATOM	841	O	ALA	A	730	57.690	11.803	38.821	1.00	24.08	A	ATOM	892	N	TYR	A	737	69.507	13.476	35.779	1.00	24.37	A
ATOM	842	N	PHE	A	731	56.603	11.102	40.665	1.00	27.43	A	ATOM	893	CA	TYR	A	737	70.517	13.927	34.824	1.00	23.86	A
ATOM	843	CA	PHE	A	731	57.787	11.157	41.508	1.00	27.52	A	ATOM	894	CB	TYR	A	737	70.442	13.080	33.543	1.00	24.42	A
ATOM	844	CB	PHE	A	731	57.506	10.537	42.873	1.00	28.62	A	ATOM	895	CG	TYR	A	737	70.970	11.662	33.695	1.00	23.36	A
ATOM	845	CG	PHE	A	731	58.743	10.343	43.709	1.00	29.64	A	ATOM	896	CD1	TYR	A	737	72.317	11.366	33.466	1.00	20.88	A
ATOM	846	CD1	PHE	A	731	59.380	11.433	44.297	1.00	28.09	A	ATOM	897	CE1	TYR	A	737	72.810	10.075	33.627	1.00	22.06	A
ATOM	847	CD2	PHE	A	731	59.284	9.069	43.889	1.00	30.41	A	ATOM	898	CD2	TYR	A	737	70.126	10.624	34.088	1.00	22.13	A
ATOM	848	CE1	PHE	A	731	60.539	11.261	45.053	1.00	28.53	A	ATOM	899	CE2	TYR	A	737	70.603	9.333	34.253	1.00	23.38	A
ATOM	849	CE2	PHE	A	731	60.443	8.882	44.643	1.00	30.45	A	ATOM	900	CZ	TYR	A	737	71.946	9.058	34.024	1.00	23.66	A
ATOM	850	CZ	PHE	A	731	61.073	9.983	45.227	1.00	30.54	A	ATOM	901	OH	TYR	A	737	72.418	7.768	34.203	1.00	22.59	A
ATOM	851	C	PHE	A	731	58.932	10.393	40.865	1.00	28.41	A	ATOM	902	C	TYR	A	737	70.306	15.416	34.488	1.00	24.65	A
ATOM	852	O	PHE	A	731	60.057	10.889	40.781	1.00	28.87	A	ATOM	903	O	TYR	A	737	71.188	16.060	33.915	1.00	23.29	A
ATOM	853	N	GLU	A	732	58.631	9.184	40.404	1.00	28.59	A	ATOM	904	N	ARG	A	738	69.138	15.954	34.851	1.00	25.77	A
ATOM	854	CA	GLU	A	732	59.634	8.322	39.797	1.00	30.40	A	ATOM	905	CA	ARG	A	738	68.803	17.362	34.599	1.00	28.28	A
ATOM	855	CB	GLU	A	732	59.098	6.901	39.710	1.00	32.43	A	ATOM	906	CB	ARG	A	738	67.401	17.497	33.992	1.00	29.01	A
ATOM	856	CG	GLU	A	732	58.510	6.403	41.006	1.00	37.94	A	ATOM	907	CG	ARG	A	738	67.271	16.992	32.594	1.00	31.95	A
ATOM	857	CD	GLU	A	732	57.979	4.995	40.878	1.00	42.59	A	ATOM	908	CD	ARG	A	738	65.840	17.073	32.124	1.00	35.67	A
ATOM	858	OE1	GLU	A	732	58.725	4.050	41.206	1.00	46.82	A	ATOM	909	NE	ARG	A	738	65.743	16.720	30.713	1.00	39.40	A
ATOM	859	OE2	GLU	A	732	56.822	4.831	40.431	1.00	43.71	A	ATOM	910	CZ	ARG	A	738	64.649	16.234	30.137	1.00	40.42	A
ATOM	860	C	GLU	A	732	60.137	8.759	38.426	1.00	30.21	A	ATOM	911	NH1	ARG	A	738	63.551	16.039	30.854	1.00	39.50	A
ATOM	861	O	GLU	A	732	61.120	8.213	37.934	1.00	31.71	A	ATOM	912	NH2	ARG	A	738	64.660	15.933	28.845	1.00	41.75	A
ATOM	862	N	LYS	A	733	59.470	9.729	37.808	1.00	28.77	A	ATOM	913	C	ARG	A	738	68.826	18.192	35.868	1.00	28.26	A
ATOM	863	CA	LYS	A	733	59.892	10.212	36.498	1.00	27.63	A	ATOM	914	O	ARG	A	738	68.657	17.668	36.963	1.00	29.03	A
ATOM	864	CB	LYS	A	733	58.663	10.534	35.628	1.00	30.09	A	ATOM	915	N	GLU	A	739	69.006	19.498	35.715	1.00	30.82	A

ATOM	916	CA	GLU A 739	69.025	20.380	36.872	1.00	32.44	A	ATOM	967	OG1	THR A 746	63.666	16.660	47.953	1.00	28.63	A
ATOM	917	CB	GLU A 739	69.483	21.794	36.473	1.00	38.00	A	ATOM	968	CG2	THR A 746	61.952	17.942	49.057	1.00	27.57	A
ATOM	918	CG	GLU A 739	68.570	22.511	35.479	1.00	44.10	A	ATOM	969	C	THR A 746	59.978	16.862	47.246	1.00	21.97	A
ATOM	919	CD	GLU A 739	68.920	23.989	35.303	1.00	50.42	A	ATOM	970	O	THR A 746	59.216	16.460	48.133	1.00	21.11	A
ATOM	920	OE1	GLU A 739	70.123	24.310	35.159	1.00	53.02	A	ATOM	971	N	TYR A 747	59.612	17.754	46.333	1.00	20.35	A
ATOM	921	OE2	GLU A 739	67.990	24.833	35.297	1.00	50.97	A	ATOM	972	CA	TYR A 747	58.256	18.296	46.333	1.00	21.23	A
ATOM	922	C	GLU A 739	67.625	20.446	37.483	1.00	31.24	A	ATOM	973	CB	TYR A 747	58.093	19.362	45.243	1.00	20.55	A
ATOM	923	O	GLU A 739	66.620	20.246	36.791	1.00	28.15	A	ATOM	974	CG	TYR A 747	56.657	19.775	45.036	1.00	19.85	A
ATOM	924	N	GLY A 740	67.574	20.707	38.788	1.00	30.46	A	ATOM	975	CD1	TYR A 747	55.941	20.421	46.047	1.00	20.82	A
ATOM	925	CA	GLY A 740	66.305	20.822	39.481	1.00	27.62	A	ATOM	976	CE1	TYR A 747	54.587	20.732	45.890	1.00	20.17	A
ATOM	926	C	GLY A 740	65.737	19.513	39.982	1.00	25.73	A	ATOM	977	CD2	TYR A 747	55.986	19.456	43.855	1.00	21.72	A
ATOM	927	O	GLY A 740	64.646	19.485	40.549	1.00	23.75	A	ATOM	978	CE2	TYR A 747	54.629	19.763	43.684	1.00	22.33	A
ATOM	928	N	TYR A 741	66.480	18.430	39.784	1.00	25.93	A	ATOM	979	CZ	TYR A 747	53.939	20.397	44.703	1.00	23.16	A
ATOM	929	CA	TYR A 741	66.031	17.108	40.201	1.00	25.36	A	ATOM	980	OH	TYR A 747	52.605	20.687	44.536	1.00	24.51	A
ATOM	930	CB	TYR A 741	67.096	16.061	39.893	1.00	25.60	A	ATOM	981	C	TYR A 747	57.229	17.179	46.108	1.00	22.58	A
ATOM	931	CG	TYR A 741	66.790	14.724	40.521	1.00	24.98	A	ATOM	982	O	TYR A 747	56.264	17.049	46.861	1.00	22.79	A
ATOM	932	CD1	TYR A 741	65.804	13.893	39.996	1.00	24.91	A	ATOM	983	N	LEU A 748	57.441	16.372	45.075	1.00	22.57	A
ATOM	933	CE1	TYR A 741	65.503	12.672	40.592	1.00	25.69	A	ATOM	984	CA	LEU A 748	56.517	15.285	44.775	1.00	24.82	A
ATOM	934	CD2	TYR A 741	67.469	14.303	41.664	1.00	26.57	A	ATOM	985	CB	LEU A 748	56.893	14.610	43.449	1.00	22.43	A
ATOM	935	CE2	TYR A 741	67.175	13.089	42.268	1.00	26.65	A	ATOM	986	CG	LEU A 748	56.729	15.521	42.230	1.00	24.29	A
ATOM	936	CZ	TYR A 741	66.194	12.278	41.726	1.00	26.81	A	ATOM	987	CD1	LEU A 748	57.124	14.781	40.956	1.00	22.32	A
ATOM	937	OH	TYR A 741	65.916	11.072	42.316	1.00	30.67	A	ATOM	988	CD2	LEU A 748	55.275	16.003	42.158	1.00	25.26	A
ATOM	938	C	TYR A 741	65.659	16.999	41.673	1.00	26.21	A	ATOM	989	C	LEU A 748	56.461	14.252	45.896	1.00	25.03	A
ATOM	939	O	TYR A 741	64.547	16.586	42.002	1.00	27.92	A	ATOM	990	O	LEU A 748	55.400	13.700	46.176	1.00	25.54	A
ATOM	940	N	ALA A 742	66.593	17.347	42.554	1.00	26.32	A	ATOM	991	N	ALA A 749	57.599	13.989	46.532	1.00	25.32	A
ATOM	941	CA	ALA A 742	66.356	17.266	43.999	1.00	25.29	A	ATOM	992	CA	ALA A 749	57.634	13.026	47.627	1.00	26.50	A
ATOM	942	CB	ALA A 742	67.586	17.754	44.760	1.00	24.78	A	ATOM	993	CB	ALA A 749	59.072	12.760	48.052	1.00	23.33	A
ATOM	943	C	ALA A 742	65.126	18.036	44.466	1.00	23.60	A	ATOM	994	C	ALA A 749	56.824	13.581	48.800	1.00	27.01	A
ATOM	944	O	ALA A 742	64.324	17.515	45.246	1.00	23.24	A	ATOM	995	O	ALA A 749	56.115	12.842	49.483	1.00	28.87	A
ATOM	945	N	ASP A 743	64.981	19.270	43.991	1.00	23.09	A	ATOM	996	N	ASP A 750	56.924	14.886	49.027	1.00	28.18	A
ATOM	946	CA	ASP A 743	63.853	20.116	44.367	1.00	23.94	A	ATOM	997	CA	ASP A 750	56.179	15.500	50.110	1.00	28.86	A
ATOM	947	CB	ASP A 743	64.033	21.526	43.772	1.00	27.09	A	ATOM	998	CB	ASP A 750	56.661	16.923	50.373	1.00	35.12	A
ATOM	948	CG	ASP A 743	62.856	22.472	44.078	1.00	32.95	A	ATOM	999	CG	ASP A 750	56.025	17.519	51.613	1.00	41.43	A
ATOM	949	OD1	ASP A 743	62.475	22.634	45.261	1.00	36.18	A	ATOM	1000	OD1	ASP A 750	56.084	16.861	52.675	1.00	45.64	A
ATOM	950	OD2	ASP A 743	62.314	23.079	43.128	1.00	34.04	A	ATOM	1001	OD2	ASP A 750	55.464	18.634	51.536	1.00	47.70	A
ATOM	951	C	ASP A 743	62.521	19.517	43.920	1.00	23.33	A	ATOM	1002	C	ASP A 750	54.693	15.524	49.778	1.00	27.50	A
ATOM	952	O	ASP A 743	61.560	19.501	44.689	1.00	23.31	A	ATOM	1003	O	ASP A 750	53.850	15.381	50.666	1.00	24.43	A
ATOM	953	N	CYS A 744	62.464	18.998	42.695	1.00	22.23	A	ATOM	1004	N	VAL A 751	54.372	15.718	48.500	1.00	24.27	A
ATOM	954	CA	CYS A 744	61.215	18.437	42.188	1.00	22.53	A	ATOM	1005	CA	VAL A 751	52.983	15.735	48.075	1.00	23.53	A
ATOM	955	CB	CYS A 744	61.271	18.248	40.668	1.00	22.28	A	ATOM	1006	CB	VAL A 751	52.849	16.086	46.559	1.00	23.05	A
ATOM	956	SG	CYS A 744	59.609	18.001	39.934	1.00	27.98	A	ATOM	1007	CG1	VAL A 751	51.488	15.671	46.026	1.00	19.64	A
ATOM	957	C	CYS A 744	60.828	17.117	42.847	1.00	20.85	A	ATOM	1008	CG2	VAL A 751	53.026	17.583	46.359	1.00	23.39	A
ATOM	958	O	CYS A 744	59.668	16.918	43.211	1.00	18.23	A	ATOM	1009	C	VAL A 751	52.396	14.348	48.347	1.00	24.65	A
ATOM	959	N	ALA A 745	61.794	16.214	42.986	1.00	21.66	A	ATOM	1010	O	VAL A 751	51.322	14.233	48.927	1.00	24.52	A
ATOM	960	CA	ALA A 745	61.542	14.922	43.618	1.00	21.89	A	ATOM	1011	N	GLU A 752	53.111	13.301	47.948	1.00	25.90	A
ATOM	961	CB	ALA A 745	62.813	14.087	43.608	1.00	18.06	A	ATOM	1012	CA	GLU A 752	52.636	11.938	48.155	1.00	29.00	A
ATOM	962	C	ALA A 745	61.066	15.133	45.060	1.00	24.26	A	ATOM	1013	CB	GLU A 752	53.626	10.911	47.590	1.00	28.75	A
ATOM	963	O	ALA A 745	60.116	14.498	45.524	1.00	26.83	A	ATOM	1014	CG	GLU A 752	53.830	10.957	46.085	1.00	30.59	A
ATOM	964	N	THR A 746	61.736	16.036	45.764	1.00	24.82	A	ATOM	1015	CD	GLU A 752	54.488	9.691	45.553	1.00	34.47	A
ATOM	965	CA	THR A 746	61.401	16.333	47.149	1.00	24.44	A	ATOM	1016	OE1	GLU A 752	55.267	9.058	46.301	1.00	35.51	A
ATOM	966	CB	THR A 746	62.428	17.345	47.738	1.00	26.26	A	ATOM	1017	OE2	GLU A 752	54.236	9.329	44.382	1.00	36.37	A

ATOM 1018	C	GLU A 752	52.399	11.627	49.629	1.00	29.95	A	ATOM 1069	C	ASN A 759	42.928	17.973	55.739	1.00	30.35	A
ATOM 1019	O	GLU A 752	51.385	11.021	49.979	1.00	31.28	A	ATOM 1070	O	ASN A 759	41.923	17.560	55.152	1.00	28.01	A
ATOM 1020	N	GLN A 753	53.334	12.031	50.486	1.00	30.06	A	ATOM 1071	N	SER A 760	43.236	17.636	56.990	1.00	29.32	A
ATOM 1021	CA	GLN A 753	53.218	11.779	51.922	1.00	30.41	A	ATOM 1072	CA	SER A 760	42.421	16.738	57.805	1.00	29.78	A
ATOM 1022	CB	GLN A 753	54.557	12.029	52.622	1.00	32.98	A	ATOM 1073	CB	SER A 760	41.030	17.337	58.018	1.00	32.18	A
ATOM 1023	CG	GLN A 753	55.571	10.917	52.447	1.00	40.74	A	ATOM 1074	OG	SER A 760	41.130	18.617	58.615	1.00	37.41	A
ATOM 1024	CD	GLN A 753	54.961	9.537	52.651	1.00	47.09	A	ATOM 1075	C	SER A 760	42.290	15.329	57.222	1.00	28.06	A
ATOM 1025	OE1	GLN A 753	54.246	9.027	51.781	1.00	49.52	A	ATOM 1076	O	SER A 760	41.320	14.627	57.499	1.00	27.40	A
ATOM 1026	NE2	GLN A 753	55.232	8.929	53.809	1.00	49.04	A	ATOM 1077	N	GLY A 761	43.272	14.926	56.422	1.00	26.47	A
ATOM 1027	C	GLN A 753	52.138	12.597	52.628	1.00	28.86	A	ATOM 1078	CA	GLY A 761	43.253	13.608	55.817	1.00	25.20	A
ATOM 1028	O	GLN A 753	51.597	12.171	53.649	1.00	28.01	A	ATOM 1079	C	GLY A 761	42.372	13.521	54.587	1.00	25.04	A
ATOM 1029	N	SER A 754	51.830	13.770	52.096	1.00	27.04	A	ATOM 1080	O	GLY A 761	42.162	12.439	54.043	1.00	25.80	A
ATOM 1030	CA	SER A 754	50.822	14.620	52.709	1.00	29.01	A	ATOM 1081	N	ALA A 762	41.871	14.663	54.130	1.00	24.55	A
ATOM 1031	CB	SER A 754	50.949	16.046	52.174	1.00	28.62	A	ATOM 1082	CA	ALA A 762	40.991	14.696	52.967	1.00	24.74	A
ATOM 1032	OG	SER A 754	50.522	16.107	50.829	1.00	28.83	A	ATOM 1083	CB	ALA A 762	40.023	15.860	53.104	1.00	25.14	A
ATOM 1033	C	SER A 754	49.389	14.119	52.483	1.00	29.61	A	ATOM 1084	C	ALA A 762	41.663	14.757	51.588	1.00	24.21	A
ATOM 1034	O	SER A 754	48.459	14.570	53.153	1.00	30.32	A	ATOM 1085	O	ALA A 762	42.597	15.524	51.367	1.00	22.71	A
ATOM 1035	N	MET A 755	49.205	13.198	51.541	1.00	29.58	A	ATOM 1086	N	VAL A 763	41.172	13.929	50.667	1.00	23.75	A
ATOM 1036	CA	MET A 755	47.869	12.673	51.258	1.00	30.33	A	ATOM 1087	CA	VAL A 763	41.662	13.915	49.292	1.00	23.88	A
ATOM 1037	CB	MET A 755	47.851	11.991	49.891	1.00	32.71	A	ATOM 1088	CB	VAL A 763	42.461	12.630	48.963	1.00	24.21	A
ATOM 1038	CG	MET A 755	48.023	12.964	48.740	1.00	36.53	A	ATOM 1089	CG1	VAL A 763	43.818	12.696	49.656	1.00	22.78	A
ATOM 1039	SD	MET A 755	47.721	12.209	47.145	1.00	44.39	A	ATOM 1090	CG2	VAL A 763	41.697	11.387	49.400	1.00	21.64	A
ATOM 1040	CE	MET A 755	45.980	12.530	46.925	1.00	41.90	A	ATOM 1091	C	VAL A 763	40.454	14.048	48.373	1.00	24.69	A
ATOM 1041	C	MET A 755	47.367	11.710	52.331	1.00	29.15	A	ATOM 1092	O	VAL A 763	39.327	13.739	48.774	1.00	25.55	A
ATOM 1042	O	MET A 755	47.962	10.663	52.571	1.00	28.37	A	ATOM 1093	N	TYR A 764	40.682	14.525	47.152	1.00	24.76	A
ATOM 1043	N	GLY A 756	46.259	12.076	52.967	1.00	29.52	A	ATOM 1094	CA	TYR A 764	39.598	14.740	46.195	1.00	24.99	A
ATOM 1044	CA	GLY A 756	45.692	11.254	54.020	1.00	30.66	A	ATOM 1095	CB	TYR A 764	39.699	16.156	45.623	1.00	26.93	A
ATOM 1045	C	GLY A 756	46.210	11.732	55.357	1.00	32.81	A	ATOM 1096	CG	TYR A 764	39.527	17.222	46.672	1.00	27.43	A
ATOM 1046	O	GLY A 756	45.975	11.119	56.400	1.00	33.97	A	ATOM 1097	CD1	TYR A 764	38.261	17.714	46.981	1.00	28.56	A
ATOM 1047	N	LEU A 757	46.928	12.845	55.321	1.00	32.73	A	ATOM 1098	CE1	TYR A 764	38.079	18.641	47.998	1.00	31.55	A
ATOM 1048	CA	LEU A 757	47.498	13.414	56.526	1.00	34.80	A	ATOM 1099	CD2	TYR A 764	40.616	17.688	47.408	1.00	26.81	A
ATOM 1049	CB	LEU A 757	49.015	13.211	56.512	1.00	37.97	A	ATOM 1100	CE2	TYR A 764	40.446	18.615	48.434	1.00	30.12	A
ATOM 1050	CG	LEU A 757	49.819	13.733	57.702	1.00	41.42	A	ATOM 1101	CZ	TYR A 764	39.171	19.087	48.719	1.00	31.77	A
ATOM 1051	CD1	LEU A 757	49.187	13.250	59.000	1.00	42.32	A	ATOM 1102	OH	TYR A 764	38.975	20.019	49.708	1.00	37.04	A
ATOM 1052	CD2	LEU A 757	51.272	13.262	57.580	1.00	40.69	A	ATOM 1103	C	TYR A 764	39.542	13.747	45.048	1.00	25.41	A
ATOM 1053	C	LEU A 757	47.152	14.896	56.612	1.00	33.91	A	ATOM 1104	O	TYR A 764	40.538	13.512	44.365	1.00	24.60	A
ATOM 1054	O	LEU A 757	46.578	15.352	57.601	1.00	31.49	A	ATOM 1105	N	ALA A 765	38.359	13.178	44.834	1.00	26.34	A
ATOM 1055	N	MET A 758	47.496	15.641	55.566	1.00	34.43	A	ATOM 1106	CA	ALA A 765	38.154	12.220	43.754	1.00	25.95	A
ATOM 1056	CA	MET A 758	47.207	17.068	55.520	1.00	35.60	A	ATOM 1107	CB	ALA A 765	36.780	11.580	43.869	1.00	22.47	A
ATOM 1057	CB	MET A 758	48.099	17.756	54.487	1.00	39.24	A	ATOM 1108	C	ALA A 765	38.273	12.976	42.443	1.00	25.76	A
ATOM 1058	CG	MET A 758	49.575	17.743	54.835	1.00	43.53	A	ATOM 1109	O	ALA A 765	37.729	14.072	42.294	1.00	27.87	A
ATOM 1059	SD	MET A 758	49.884	18.489	56.456	1.00	51.96	A	ATOM 1110	N	LEU A 766	38.986	12.391	41.492	1.00	25.15	A
ATOM 1060	CE	MET A 758	49.429	20.218	56.126	1.00	48.27	A	ATOM 1111	CA	LEU A 766	39.189	13.024	40.203	1.00	24.67	A
ATOM 1061	C	MET A 758	45.744	17.307	55.159	1.00	34.98	A	ATOM 1112	CB	LEU A 766	40.675	12.963	39.844	1.00	21.86	A
ATOM 1062	O	MET A 758	45.047	16.395	54.698	1.00	34.34	A	ATOM 1113	CG	LEU A 766	41.611	13.582	40.891	1.00	20.15	A
ATOM 1063	N	ASN A 759	45.289	18.539	55.368	1.00	32.78	A	ATOM 1114	CD1	LEU A 766	43.056	13.409	40.463	1.00	17.28	A
ATOM 1064	CA	ASN A 759	43.918	18.924	55.059	1.00	31.74	A	ATOM 1115	CD2	LEU A 766	41.280	15.065	41.064	1.00	19.40	A
ATOM 1065	CB	ASN A 759	43.731	18.936	53.535	1.00	32.94	A	ATOM 1116	C	LEU A 766	38.347	12.339	39.129	1.00	26.19	A
ATOM 1066	CG	ASN A 759	42.463	19.647	53.104	1.00	37.37	A	ATOM 1117	O	LEU A 766	38.332	12.757	37.970	1.00	26.24	A
ATOM 1067	OD1	ASN A 759	42.044	20.626	53.723	1.00	38.82	A	ATOM 1118	N	TRP A 767	37.648	11.284	39.535	1.00	26.69	A
ATOM 1068	ND2	ASN A 759	41.853	19.169	52.024	1.00	39.45	A	ATOM 1119	CA	TRP A 767	36.786	10.505	38.644	1.00	26.68	A

ATOM 1120	CB	TRP A 767	37.590	9.423	37.926	1.00	26.20	A	ATOM 1171	O	GLU A 772	33.020	-5.886	43.503	1.00	34.86	A
ATOM 1121	CG	TRP A 767	38.624	9.913	36.974	1.00	26.84	A	ATOM 1172	N	PHE A 773	33.505	-4.341	45.075	1.00	32.37	A
ATOM 1122	CD2	TRP A 767	40.039	9.964	37.200	1.00	24.84	A	ATOM 1173	CA	PHE A 773	33.274	-5.220	46.207	1.00	29.03	A
ATOM 1123	CE2	TRP A 767	40.638	10.420	36.002	1.00	24.82	A	ATOM 1174	CB	PHE A 773	34.605	-5.798	46.695	1.00	29.15	A
ATOM 1124	CE3	TRP A 767	40.859	9.664	38.294	1.00	23.86	A	ATOM 1175	CG	PHE A 773	35.243	-6.749	45.725	1.00	28.27	A
ATOM 1125	CE4	TRP A 767	38.424	10.338	35.690	1.00	26.46	A	ATOM 1176	CD1	PHE A 773	34.754	-8.045	45.576	1.00	29.60	A
ATOM 1126	NE1	TRP A 767	39.632	10.639	35.098	1.00	26.53	A	ATOM 1177	CD2	PHE A 773	36.315	-6.341	44.932	1.00	28.82	A
ATOM 1127	CZ2	TRP A 767	42.021	10.580	35.868	1.00	23.16	A	ATOM 1178	CE1	PHE A 773	35.326	-8.925	44.648	1.00	28.13	A
ATOM 1128	CZ3	TRP A 767	42.240	9.823	38.158	1.00	24.42	A	ATOM 1179	CE2	PHE A 773	36.894	-7.209	44.002	1.00	27.51	A
ATOM 1129	CH2	TRP A 767	42.803	10.276	36.953	1.00	23.17	A	ATOM 1180	CZ	PHE A 773	36.398	-8.501	43.860	1.00	28.79	A
ATOM 1130	C	TRP A 767	35.735	9.805	39.487	1.00	27.99	A	ATOM 1181	C	PHE A 773	32.613	-4.415	47.314	1.00	29.59	A
ATOM 1131	O	TRP A 767	35.916	9.641	40.697	1.00	27.04	A	ATOM 1182	O	PHE A 773	32.785	-3.194	47.389	1.00	27.43	A
ATOM 1132	N	ASP A 768	34.639	9.390	38.852	1.00	29.80	A	ATOM 1183	N	GLY A 774	31.857	-5.105	48.167	1.00	29.44	A
ATOM 1133	CA	ASP A 768	33.596	8.655	39.568	1.00	30.48	A	ATOM 1184	CA	GLY A 774	31.171	-4.452	49.265	1.00	28.62	A
ATOM 1134	CB	ASP A 768	32.293	8.565	38.757	1.00	32.71	A	ATOM 1185	C	GLY A 774	32.109	-3.725	50.206	1.00	30.05	A
ATOM 1135	CG	ASP A 768	31.698	9.919	38.429	1.00	36.52	A	ATOM 1186	O	GLY A 774	31.728	-2.716	50.800	1.00	32.67	A
ATOM 1136	OD1	ASP A 768	31.709	10.812	39.300	1.00	37.07	A	ATOM 1187	N	ASP A 775	33.338	-4.224	50.334	1.00	30.38	A
ATOM 1137	OD2	ASP A 768	31.198	10.084	37.292	1.00	40.28	A	ATOM 1188	CA	ASP A 775	34.329	-3.615	51.219	1.00	30.45	A
ATOM 1138	C	ASP A 768	34.139	7.236	39.727	1.00	28.84	A	ATOM 1189	CB	ASP A 775	35.351	-4.666	51.669	1.00	30.28	A
ATOM 1139	O	ASP A 768	34.909	6.766	38.886	1.00	25.55	A	ATOM 1190	CG	ASP A 775	36.281	-5.089	50.555	1.00	30.13	A
ATOM 1140	N	TYR A 769	33.748	6.560	40.801	1.00	28.29	A	ATOM 1191	OD1	ASP A 775	35.852	-5.087	49.385	1.00	34.19	A
ATOM 1141	CA	TYR A 769	34.176	5.183	41.010	1.00	28.69	A	ATOM 1192	OD2	ASP A 775	37.443	-5.436	50.850	1.00	32.81	A
ATOM 1142	CG	TYR A 769	35.402	5.099	41.931	1.00	27.18	A	ATOM 1193	C	ASP A 775	35.054	-2.430	50.580	1.00	30.00	A
ATOM 1143	CB	TYR A 769	35.840	3.664	42.168	1.00	28.09	A	ATOM 1194	O	ASP A 775	36.039	-1.926	51.124	1.00	30.42	A
ATOM 1144	OD1	TYR A 769	36.350	2.888	41.120	1.00	29.26	A	ATOM 1195	N	GLU A 776	34.567	-2.001	49.421	1.00	29.92	A
ATOM 1145	CE1	TYR A 769	36.654	1.536	41.299	1.00	28.65	A	ATOM 1196	CA	GLU A 776	35.134	-0.860	48.710	1.00	29.84	A
ATOM 1146	CE2	TYR A 769	35.656	3.051	43.409	1.00	26.89	A	ATOM 1197	CB	GLU A 776	35.456	-1.222	47.258	1.00	29.22	A
ATOM 1147	CE3	TYR A 769	35.958	1.696	43.602	1.00	25.59	A	ATOM 1198	CG	GLU A 776	36.757	-1.993	47.065	1.00	29.15	A
ATOM 1148	CZ	TYR A 769	36.452	0.945	42.544	1.00	29.09	A	ATOM 1199	CD	GLU A 776	36.927	-2.478	45.639	1.00	30.60	A
ATOM 1149	OH	TYR A 769	36.716	-0.401	42.715	1.00	28.89	A	ATOM 1200	OE1	GLU A 776	36.157	-2.019	44.764	1.00	31.39	A
ATOM 1150	C	TYR A 769	33.051	4.342	41.606	1.00	28.56	A	ATOM 1201	OE2	GLU A 776	37.828	-3.309	45.387	1.00	30.56	A
ATOM 1151	O	TYR A 769	32.436	4.722	42.605	1.00	28.74	A	ATOM 1202	C	GLU A 776	34.117	0.274	48.747	1.00	30.19	A
ATOM 1152	N	SER A 770	32.779	3.203	40.982	1.00	27.30	A	ATOM 1203	O	GLU A 776	32.932	0.046	48.991	1.00	29.16	A
ATOM 1153	CA	SER A 770	31.746	2.301	41.476	1.00	28.69	A	ATOM 1204	N	LEU A 777	34.581	1.488	48.480	1.00	30.28	A
ATOM 1154	CB	SER A 770	30.827	1.843	40.342	1.00	29.89	A	ATOM 1205	CA	LEU A 777	33.726	2.669	48.527	1.00	30.17	A
ATOM 1155	OG	SER A 770	30.048	2.917	39.857	1.00	33.37	A	ATOM 1206	CB	LEU A 777	34.404	3.703	49.425	1.00	31.19	A
ATOM 1156	C	SER A 770	32.450	1.097	42.071	1.00	27.28	A	ATOM 1207	CG	LEU A 777	33.662	4.827	50.134	1.00	33.07	A
ATOM 1157	O	SER A 770	33.143	0.370	41.365	1.00	25.80	A	ATOM 1208	CD1	LEU A 777	32.583	4.267	51.064	1.00	33.14	A
ATOM 1158	N	ALA A 771	32.270	0.901	43.371	1.00	28.92	A	ATOM 1209	CD2	LEU A 777	34.684	5.630	50.930	1.00	31.96	A
ATOM 1159	CA	ALA A 771	32.892	-0.206	44.088	1.00	30.18	A	ATOM 1210	C	LEU A 777	33.462	3.268	47.141	1.00	30.47	A
ATOM 1160	CB	ALA A 771	32.334	-0.276	45.508	1.00	29.78	A	ATOM 1211	O	LEU A 777	34.370	3.348	46.306	1.00	31.11	A
ATOM 1161	C	ALA A 771	32.685	-1.536	43.370	1.00	30.95	A	ATOM 1212	N	SER A 778	32.213	3.668	46.897	1.00	28.47	A
ATOM 1162	O	ALA A 771	31.631	-1.781	42.790	1.00	32.57	A	ATOM 1213	CA	SER A 778	31.822	4.291	45.636	1.00	28.06	A
ATOM 1163	N	GLU A 772	33.705	-2.383	43.401	1.00	32.38	A	ATOM 1214	CB	SER A 778	30.391	3.891	45.248	1.00	29.13	A
ATOM 1164	CA	GLU A 772	33.643	-3.690	42.763	1.00	34.65	A	ATOM 1215	OG	SER A 778	30.289	2.504	44.975	1.00	32.82	A
ATOM 1165	CB	GLU A 772	34.971	-4.006	42.080	1.00	37.34	A	ATOM 1216	C	SER A 778	31.871	5.800	45.851	1.00	27.40	A
ATOM 1166	CG	GLU A 772	35.163	-3.306	40.747	1.00	44.96	A	ATOM 1217	O	SER A 778	31.593	6.275	46.949	1.00	27.17	A
ATOM 1167	CD	GLU A 772	36.614	-3.316	40.298	1.00	49.26	A	ATOM 1218	N	PHE A 779	32.221	6.552	44.812	1.00	26.80	A
ATOM 1168	OE1	GLU A 772	37.262	-4.381	40.422	1.00	52.75	A	ATOM 1219	CA	PHE A 779	32.291	8.002	44.928	1.00	25.92	A
ATOM 1169	OE2	GLU A 772	37.101	-2.264	39.821	1.00	48.75	A	ATOM 1220	CB	PHE A 779	33.558	8.411	45.702	1.00	23.94	A
ATOM 1170	C	GLU A 772	33.356	-4.743	43.818	1.00	33.98	A	ATOM 1221	CG	PHE A 779	34.850	7.872	45.122	1.00	21.84	A

ATOM 1222	CD1 PHE A 779	35.451	8.481	44.020	1.00	20.86	A	ATOM 1273	C	VAL A 785	38.294	11.584	51.960	1.00	25.80	A
ATOM 1223	CD2 PHE A 779	35.476	6.761	45.692	1.00	21.49	A	ATOM 1274	O	VAL A 785	39.388	12.087	51.735	1.00	25.45	A
ATOM 1224	CE1 PHE A 779	36.657	7.994	43.496	1.00	16.05	A	ATOM 1275	N	THR A 786	37.965	11.080	53.146	1.00	25.20	A
ATOM 1225	CE2 PHE A 779	36.682	6.268	45.173	1.00	19.14	A	ATOM 1276	CA	THR A 786	38.927	11.090	54.236	1.00	28.24	A
ATOM 1226	CZ PHE A 779	37.269	6.889	44.074	1.00	16.59	A	ATOM 1277	CB	THR A 786	38.274	11.300	55.615	1.00	28.16	A
ATOM 1227	C PHE A 779	32.244	8.691	43.571	1.00	28.28	A	ATOM 1278	CG1 THR A 786	37.527	12.518	55.617	1.00	29.45	A	
ATOM 1228	O PHE A 779	32.533	8.076	42.545	1.00	29.15	A	ATOM 1279	CG2 THR A 786	39.356	11.383	56.693	1.00	28.17	A	
ATOM 1229	N ARG A 780	31.861	9.965	43.568	1.00	29.80	A	ATOM 1280	C	THR A 786	39.656	9.764	54.274	1.00	29.65	A
ATOM 1230	CA ARG A 780	31.783	10.739	42.327	1.00	32.70	A	ATOM 1281	O	THR A 786	39.032	8.699	54.265	1.00	30.31	A
ATOM 1231	CB ARG A 780	30.472	11.532	42.249	1.00	34.69	A	ATOM 1282	N	VAL A 787	40.981	9.841	54.322	1.00	29.84	A
ATOM 1232	CG ARG A 780	29.180	10.724	42.318	1.00	39.68	A	ATOM 1283	CA	VAL A 787	41.812	8.654	54.365	1.00	31.77	A
ATOM 1233	CD ARG A 780	29.077	9.657	41.228	1.00	43.29	A	ATOM 1284	CB	VAL A 787	43.184	8.910	53.714	1.00	30.29	A
ATOM 1234	NE ARG A 780	29.571	8.358	41.689	1.00	47.15	A	ATOM 1285	CG1 VAL A 787	44.030	7.654	53.790	1.00	29.26	A	
ATOM 1235	CZ ARG A 780	29.491	7.233	40.983	1.00	48.18	A	ATOM 1286	CG2 VAL A 787	42.998	9.344	52.271	1.00	28.98	A	
ATOM 1236	NH1 ARG A 780	28.932	7.246	39.777	1.00	49.30	A	ATOM 1287	C	VAL A 787	42.022	8.229	55.806	1.00	33.74	A
ATOM 1237	NH2 ARG A 780	29.970	6.097	41.481	1.00	46.34	A	ATOM 1288	O	VAL A 787	42.685	8.924	56.577	1.00	33.28	A
ATOM 1238	C ARG A 780	32.943	11.727	42.218	1.00	32.15	A	ATOM 1289	N	LEU A 788	41.451	7.082	56.160	1.00	36.49	A
ATOM 1239	O ARG A 780	33.565	12.088	43.218	1.00	32.88	A	ATOM 1290	CA	LEU A 788	41.558	6.549	57.511	1.00	38.77	A
ATOM 1240	N	33.213	12.166	40.994	1.00	32.79	A	ATOM 1291	CB	LEU A 788	40.325	5.694	57.832	1.00	37.66	A
ATOM 1241	CA	34.276	13.124	40.713	1.00	33.17	A	ATOM 1292	CG	LEU A 788	38.979	6.430	57.742	1.00	37.88	A
ATOM 1242	CB	34.315	13.412	39.205	1.00	35.05	A	ATOM 1293	CD1 LEU A 788	37.827	5.467	58.015	1.00	35.00	A	
ATOM 1243	CG	35.355	14.427	38.736	1.00	38.87	A	ATOM 1294	CD2 LEU A 788	38.964	7.582	58.739	1.00	34.68	A	
ATOM 1244	OE1	34.930	15.876	38.960	1.00	42.86	A	ATOM 1295	C	LEU A 788	42.832	5.728	57.686	1.00	41.07	A
ATOM 1245	OE2	33.795	16.238	38.582	1.00	44.27	A	ATOM 1296	O	LEU A 788	43.614	5.974	58.603	1.00	41.19	A
ATOM 1246	C	35.736	16.663	39.506	1.00	45.87	A	ATOM 1297	N	ARG A 789	43.039	4.754	56.806	1.00	44.78	A
ATOM 1247	GLU A 781	34.013	14.405	41.490	1.00	32.96	A	ATOM 1298	CA	ARG A 789	44.228	3.908	56.874	1.00	49.14	A
ATOM 1248	GLU A 781	32.887	14.896	41.530	1.00	32.18	A	ATOM 1299	CB	ARG A 789	43.840	2.444	57.123	1.00	50.34	A
ATOM 1249	N	35.055	14.932	42.126	1.00	34.02	A	ATOM 1300	CG	ARG A 789	43.235	2.183	58.498	1.00	53.78	A
ATOM 1250	CA	34.913	16.160	42.884	1.00	31.39	A	ATOM 1301	CD	ARG A 789	42.923	0.700	58.717	1.00	56.18	A
ATOM 1251	C	34.623	15.995	44.363	1.00	31.32	A	ATOM 1302	NE	ARG A 789	41.857	0.204	57.846	1.00	57.68	A
ATOM 1252	O	34.691	16.964	45.120	1.00	31.10	A	ATOM 1303	CZ	ARG A 789	41.456	-1.065	57.801	1.00	59.18	A
ATOM 1253	N	34.311	14.782	44.799	1.00	31.35	A	ATOM 1304	NH1	ARG A 789	42.031	-1.974	58.576	1.00	61.30	A
ATOM 1254	CA	34.011	14.581	46.212	1.00	31.55	A	ATOM 1305	NH2	ARG A 789	40.475	-1.429	56.985	1.00	59.42	A
ATOM 1255	CB	33.097	13.370	46.393	1.00	33.95	A	ATOM 1306	C	ARG A 789	45.022	4.016	55.582	1.00	50.65	A
ATOM 1256	CG	31.724	13.589	45.773	1.00	40.97	A	ATOM 1307	O	ARG A 789	44.578	3.572	54.526	1.00	50.71	A
ATOM 1257	CD	30.728	12.593	46.122	1.00	44.21	A	ATOM 1308	N	ARG A 790	46.205	4.611	55.678	1.00	53.92	A
ATOM 1258	OE1	30.961	11.329	45.751	1.00	44.53	A	ATOM 1309	CA	ARG A 790	47.071	4.798	54.523	1.00	56.88	A
ATOM 1259	OE2	29.710	12.834	46.771	1.00	47.71	A	ATOM 1310	CB	ARG A 790	47.888	6.076	54.681	1.00	58.15	A
ATOM 1260	C	35.242	14.448	47.097	1.00	29.63	A	ATOM 1311	CG	ARG A 790	47.129	7.366	54.497	1.00	61.19	A
ATOM 1261	O	36.287	13.984	46.667	1.00	29.71	A	ATOM 1312	CD	ARG A 790	48.041	8.537	54.825	1.00	63.65	A
ATOM 1262	N	35.095	14.876	48.342	1.00	29.02	A	ATOM 1313	NE	ARG A 790	49.391	8.323	54.304	1.00	66.00	A
ATOM 1263	CA	36.164	14.834	49.330	1.00	29.55	A	ATOM 1314	CZ	ARG A 790	50.423	9.127	54.546	1.00	66.55	A
ATOM 1264	CB	36.023	16.028	50.287	1.00	28.25	A	ATOM 1315	NH1	ARG A 790	50.264	10.207	55.300	1.00	67.17	A
ATOM 1265	OG	36.938	15.940	51.361	1.00	27.34	A	ATOM 1316	NH2	ARG A 790	51.618	8.841	54.047	1.00	66.16	A
ATOM 1266	C	36.091	13.528	50.114	1.00	28.41	A	ATOM 1317	C	ARG A 790	48.042	3.653	54.308	1.00	58.33	A
ATOM 1267	O	35.053	13.199	50.677	1.00	30.24	A	ATOM 1318	O	ARG A 790	47.969	2.929	53.318	1.00	59.18	A
ATOM 1268	N	37.192	12.787	50.144	1.00	28.12	A	ATOM 1319	N	ASP A 791	48.962	3.510	55.250	1.00	59.70	A
ATOM 1269	CA	37.241	11.518	50.862	1.00	26.67	A	ATOM 1320	CA	ASP A 791	49.996	2.496	55.173	1.00	61.32	A
ATOM 1270	CB	37.596	10.342	49.907	1.00	25.83	A	ATOM 1321	CB	ASP A 791	51.170	2.923	56.053	1.00	63.34	A
ATOM 1271	CG1	37.797	9.067	50.699	1.00	24.98	A	ATOM 1322	CG	ASP A 791	51.462	4.413	55.940	1.00	65.14	A
ATOM 1272	CG2	36.493	10.139	48.894	1.00	25.69	A	ATOM 1323	OD1	ASP A 791	51.599	4.909	54.798	1.00	65.74	A

ATOM 1324	OD2	ASP A 791	51.550	5.089	56.990	1.00	65.92	A	ATOM 1375	CD2	TRP A 798	41.914	-0.549	45.991	1.00	24.53	A
ATOM 1325	C	ASP A 791	49.538	1.091	55.544	1.00	61.34	A	ATOM 1376	CE2	TRP A 798	42.185	-1.248	44.789	1.00	24.61	A
ATOM 1326	O	ASP A 791	50.316	0.305	56.087	1.00	61.72	A	ATOM 1377	CE3	TRP A 798	40.633	-0.008	46.188	1.00	25.12	A
ATOM 1327	N	GLY A 792	48.278	0.778	55.253	1.00	60.69	A	ATOM 1378	CD1	TRP A 798	44.037	-1.261	46.037	1.00	24.49	A
ATOM 1328	CA	GLY A 792	47.777	-0.551	55.546	1.00	60.18	A	ATOM 1379	NE1	TRP A 798	43.486	-1.671	44.844	1.00	23.87	A
ATOM 1329	C	GLY A 792	48.592	-1.537	54.728	1.00	60.34	A	ATOM 1380	CZ2	TRP A 798	41.221	-1.420	43.785	1.00	24.16	A
ATOM 1330	O	GLY A 792	48.674	-1.401	53.508	1.00	61.05	A	ATOM 1381	CZ3	TRP A 798	39.670	-0.180	45.187	1.00	23.45	A
ATOM 1331	N	PRO A 793	49.217	-2.538	55.366	1.00	59.94	A	ATOM 1382	CH2	TRP A 798	39.973	-0.882	44.000	1.00	24.45	A
ATOM 1332	CD	PRO A 793	49.217	-2.815	56.815	1.00	60.58	A	ATOM 1383	C	TRP A 798	44.413	1.814	49.565	1.00	28.43	A
ATOM 1333	CA	PRO A 793	50.028	-3.527	54.649	1.00	58.65	A	ATOM 1384	O	TRP A 798	45.355	1.518	50.301	1.00	27.53	A
ATOM 1334	CB	PRO A 793	50.716	-4.286	55.781	1.00	58.90	A	ATOM 1385	N	TRP A 799	43.453	2.661	49.930	1.00	27.07	A
ATOM 1335	CG	PRO A 793	49.679	-4.258	56.863	1.00	59.90	A	ATOM 1386	CA	TRP A 799	43.417	3.253	51.264	1.00	27.67	A
ATOM 1336	C	PRO A 793	49.261	-4.459	53.710	1.00	57.21	A	ATOM 1387	CB	TRP A 799	43.610	4.776	51.237	1.00	28.97	A
ATOM 1337	O	PRO A 793	49.795	-4.880	52.681	1.00	57.51	A	ATOM 1388	CG	TRP A 799	44.924	5.286	50.752	1.00	31.34	A
ATOM 1338	N	GLU A 794	48.019	-4.784	54.060	1.00	54.60	A	ATOM 1389	CD2	TRP A 799	45.136	6.461	49.960	1.00	29.70	A
ATOM 1339	CA	GLU A 794	47.221	-5.684	53.233	1.00	52.17	A	ATOM 1390	CE2	TRP A 799	46.528	6.608	49.792	1.00	31.24	A
ATOM 1340	CB	GLU A 794	45.929	-6.076	53.961	1.00	53.69	A	ATOM 1391	CE3	TRP A 799	44.281	7.405	49.377	1.00	30.12	A
ATOM 1341	CG	GLU A 794	45.214	-4.949	54.698	1.00	56.43	A	ATOM 1392	CD1	TRP A 799	46.162	4.779	51.026	1.00	33.36	A
ATOM 1342	CD	GLU A 794	45.932	-4.529	55.970	1.00	56.99	A	ATOM 1393	NE1	TRP A 799	47.132	5.569	50.452	1.00	33.74	A
ATOM 1343	OE1	GLU A 794	46.701	-3.546	55.928	1.00	57.66	A	ATOM 1394	CZ2	TRP A 799	47.090	7.663	49.060	1.00	30.17	A
ATOM 1344	OE2	GLU A 794	45.731	-5.191	57.012	1.00	57.57	A	ATOM 1395	CZ3	TRP A 799	44.838	8.455	48.649	1.00	30.53	A
ATOM 1345	C	GLU A 794	46.898	-5.129	51.845	1.00	49.58	A	ATOM 1396	CH2	TRP A 799	46.232	8.572	48.499	1.00	28.93	A
ATOM 1346	O	GLU A 794	46.578	-5.883	50.923	1.00	47.47	A	ATOM 1397	C	TRP A 799	42.050	3.008	51.872	1.00	26.41	A
ATOM 1347	N	GLU A 795	47.005	-3.815	51.689	1.00	46.55	A	ATOM 1398	O	TRP A 799	41.047	2.990	51.160	1.00	23.76	A
ATOM 1348	CA	GLU A 795	46.716	-3.189	50.410	1.00	44.35	A	ATOM 1399	N	TRP A 800	42.008	2.828	53.187	1.00	27.09	A
ATOM 1349	CB	GLU A 795	45.243	-2.764	50.382	1.00	43.86	A	ATOM 1400	CA	TRP A 800	40.730	2.647	53.863	1.00	27.46	A
ATOM 1350	CG	GLU A 795	44.583	-2.855	49.023	1.00	42.71	A	ATOM 1401	CB	TRP A 800	40.921	1.986	55.224	1.00	27.89	A
ATOM 1351	CD	GLU A 795	44.490	-4.280	48.501	1.00	42.79	A	ATOM 1402	CG	TRP A 800	39.619	1.588	55.836	1.00	31.41	A
ATOM 1352	OE1	GLU A 795	43.658	-5.062	49.010	1.00	40.13	A	ATOM 1403	CD2	TRP A 800	38.751	0.537	55.392	1.00	31.13	A
ATOM 1353	OE2	GLU A 795	45.258	-4.615	47.575	1.00	43.44	A	ATOM 1404	CE2	TRP A 800	37.606	0.564	56.216	1.00	32.13	A
ATOM 1354	C	GLU A 795	47.634	-1.981	50.215	1.00	43.90	A	ATOM 1405	CE3	TRP A 800	38.832	-0.425	54.375	1.00	32.31	A
ATOM 1355	O	GLU A 795	47.462	-0.932	50.865	1.00	44.71	A	ATOM 1406	CD1	TRP A 800	38.982	2.192	56.886	1.00	30.92	A
ATOM 1356	N	THR A 796	48.611	-2.105	49.322	1.00	42.63	A	ATOM 1407	NE1	TRP A 800	37.774	1.583	57.119	1.00	31.53	A
ATOM 1357	CA	THR A 796	49.545	-1.009	49.068	1.00	41.28	A	ATOM 1408	CZ2	TRP A 800	36.545	-0.334	56.056	1.00	32.52	A
ATOM 1358	CB	THR A 796	50.923	-1.542	48.603	1.00	43.16	A	ATOM 1409	CZ3	TRP A 800	37.780	-1.318	54.215	1.00	33.37	A
ATOM 1359	OG1	THR A 796	50.775	-2.280	47.380	1.00	46.47	A	ATOM 1410	CH2	TRP A 800	36.649	-1.265	55.054	1.00	33.25	A
ATOM 1360	CG2	THR A 796	51.526	-2.449	49.666	1.00	43.90	A	ATOM 1411	C	TRP A 800	40.223	4.077	54.016	1.00	25.65	A
ATOM 1361	C	THR A 796	49.051	0.026	48.046	1.00	39.43	A	ATOM 1412	O	TRP A 800	40.931	4.931	54.539	1.00	25.29	A
ATOM 1362	O	THR A 796	49.555	1.148	48.008	1.00	39.11	A	ATOM 1413	N	ALA A 801	39.012	4.351	53.545	1.00	25.43	A
ATOM 1363	N	ASP A 797	48.067	-0.338	47.228	1.00	35.46	A	ATOM 1414	CA	ALA A 801	38.501	5.716	53.604	1.00	25.89	A
ATOM 1364	CA	ASP A 797	47.549	0.589	46.228	1.00	33.17	A	ATOM 1415	CB	ALA A 801	38.669	6.367	52.241	1.00	26.31	A
ATOM 1365	CB	ASP A 797	47.574	-0.063	44.848	1.00	33.68	A	ATOM 1416	C	ALA A 801	37.062	5.874	54.075	1.00	26.70	A
ATOM 1366	CG	ASP A 797	48.987	-0.284	44.337	1.00	36.59	A	ATOM 1417	O	ALA A 801	36.350	4.889	54.278	1.00	26.56	A
ATOM 1367	OD1	ASP A 797	49.757	0.705	44.245	1.00	36.55	A	ATOM 1418	N	ALA A 802	36.643	7.132	54.230	1.00	26.25	A
ATOM 1368	CD2	ASP A 797	49.328	-1.445	44.027	1.00	37.42	A	ATOM 1419	CA	ALA A 802	35.295	7.462	54.686	1.00	25.52	A
ATOM 1369	C	ASP A 797	46.144	1.104	46.533	1.00	31.50	A	ATOM 1420	CB	ALA A 802	35.338	7.942	56.133	1.00	24.83	A
ATOM 1370	O	ASP A 797	45.528	1.774	45.705	1.00	29.17	A	ATOM 1421	C	ALA A 802	34.618	8.520	53.828	1.00	26.79	A
ATOM 1371	N	TRP A 798	45.643	0.784	47.722	1.00	29.46	A	ATOM 1422	O	ALA A 802	35.200	9.564	53.531	1.00	28.39	A
ATOM 1372	CA	TRP A 798	44.319	1.231	48.146	1.00	28.51	A	ATOM 1423	N	LEU A 803	33.383	8.241	53.432	1.00	27.11	A
ATOM 1373	CB	TRP A 798	43.323	0.059	48.131	1.00	25.76	A	ATOM 1424	CA	LEU A 803	32.594	9.169	52.631	1.00	28.25	A
ATOM 1374	CG	TRP A 798	43.116	-0.569	46.775	1.00	24.47	A	ATOM 1425	CB	LEU A 803	32.633	8.785	51.147	1.00	28.39	A

ATOM 1426	CG	LEU A 803	31.771	9.619	50.188	1.00	29.29	A	ATOM 1477	C	TYR A 809	38.528	1.742	48.936	1.00	23.91	A
ATOM 1427	CD1	LEU A 803	31.966	11.110	50.443	1.00	29.40	A	ATOM 1478	O	TYR A 809	37.506	1.643	48.263	1.00	23.77	A
ATOM 1428	CD2	LEU A 803	32.138	9.276	48.754	1.00	29.86	A	ATOM 1479	N	VAL A 810	39.523	2.564	48.623	1.00	23.47	A
ATOM 1429	C	LEU A 803	31.161	9.125	53.152	1.00	29.46	A	ATOM 1480	CA	VAL A 810	39.478	3.389	47.425	1.00	22.39	A
ATOM 1430	O	LEU A 803	30.473	8.110	53.020	1.00	26.29	A	ATOM 1481	CB	VAL A 810	39.105	4.860	47.748	1.00	22.97	A
ATOM 1431	N	HIS A 804	30.731	10.232	53.761	1.00	31.80	A	ATOM 1482	CG1	VAL A 810	37.714	4.916	48.361	1.00	22.07	A
ATOM 1432	CA	HIS A 804	29.394	10.349	54.327	1.00	32.45	A	ATOM 1483	CG2	VAL A 810	40.141	5.478	48.681	1.00	20.00	A
ATOM 1433	CB	HIS A 804	28.340	10.351	53.215	1.00	35.29	A	ATOM 1484	C	VAL A 810	40.854	3.342	46.775	1.00	21.52	A
ATOM 1434	CG	HIS A 804	28.393	11.559	52.333	1.00	36.92	A	ATOM 1485	O	VAL A 810	41.876	3.295	47.468	1.00	22.99	A
ATOM 1435	CD2	HIS A 804	29.216	12.635	52.342	1.00	37.49	A	ATOM 1486	N	PRO A 811	40.901	3.335	45.433	1.00	18.87	A
ATOM 1436	CD1	HIS A 804	27.525	11.750	51.280	1.00	39.40	A	ATOM 1487	CD	PRO A 811	39.773	3.314	44.482	1.00	18.17	A
ATOM 1437	CE1	HIS A 804	27.812	12.890	50.677	1.00	41.27	A	ATOM 1488	CA	PRO A 811	42.185	3.290	44.733	1.00	18.31	A
ATOM 1438	NE2	HIS A 804	28.834	13.447	51.302	1.00	39.38	A	ATOM 1489	CB	PRO A 811	41.781	2.920	43.305	1.00	17.47	A
ATOM 1439	C	HIS A 804	29.095	9.226	55.316	1.00	32.85	A	ATOM 1490	CG	PRO A 811	40.450	3.587	43.153	1.00	19.20	A
ATOM 1440	O	HIS A 804	28.022	8.618	55.271	1.00	33.77	A	ATOM 1491	C	PRO A 811	42.922	4.627	44.822	1.00	19.78	A
ATOM 1441	N	GLY A 805	30.051	8.942	56.197	1.00	32.12	A	ATOM 1492	O	PRO A 811	42.365	5.681	44.509	1.00	18.97	A
ATOM 1442	CA	GLY A 805	29.847	7.907	57.196	1.00	31.63	A	ATOM 1493	N	ARG A 812	44.176	4.574	45.251	1.00	21.02	A
ATOM 1443	C	GLY A 805	30.120	6.488	56.740	1.00	32.83	A	ATOM 1494	CA	ARG A 812	44.997	5.770	45.400	1.00	22.66	A
ATOM 1444	O	GLY A 805	30.171	5.568	57.563	1.00	32.31	A	ATOM 1495	CB	ARG A 812	46.417	5.388	45.807	1.00	23.98	A
ATOM 1445	N	GLN A 806	30.285	6.293	55.436	1.00	33.11	A	ATOM 1496	CG	ARG A 812	46.516	4.621	47.092	1.00	27.90	A
ATOM 1446	CA	GLN A 806	30.568	4.959	54.919	1.00	34.42	A	ATOM 1497	CD	ARG A 812	47.925	4.704	47.644	1.00	27.73	A
ATOM 1447	CB	GLN A 806	29.968	4.771	53.522	1.00	34.05	A	ATOM 1498	NE	ARG A 812	48.945	4.217	46.721	1.00	26.97	A
ATOM 1448	CG	GLN A 806	28.452	4.881	53.455	1.00	36.81	A	ATOM 1499	CZ	ARG A 812	50.239	4.161	47.021	1.00	29.21	A
ATOM 1449	CD	GLN A 806	27.738	3.972	54.451	1.00	37.82	A	ATOM 1500	NH1	ARG A 812	50.655	4.567	48.214	1.00	28.55	A
ATOM 1450	OE1	GLN A 806	28.037	2.777	54.558	1.00	37.87	A	ATOM 1501	NH2	ARG A 812	51.113	3.690	46.141	1.00	29.94	A
ATOM 1451	NE2	GLN A 806	26.775	4.539	55.176	1.00	36.39	A	ATOM 1502	C	ARG A 812	45.098	6.687	44.184	1.00	23.27	A
ATOM 1452	C	GLN A 806	32.075	4.747	54.851	1.00	34.36	A	ATOM 1503	O	ARG A 812	45.083	7.915	44.337	1.00	23.61	A
ATOM 1453	O	GLN A 806	32.821	5.654	54.480	1.00	32.67	A	ATOM 1504	N	ASN A 813	45.202	6.102	42.992	1.00	20.75	A
ATOM 1454	N	GLU A 807	32.520	3.549	55.216	1.00	34.01	A	ATOM 1505	CA	ASN A 813	45.366	6.882	41.767	1.00	22.23	A
ATOM 1455	CA	GLU A 807	33.938	3.227	55.173	1.00	34.35	A	ATOM 1506	CB	ASN A 813	45.899	5.990	40.635	1.00	23.64	A
ATOM 1456	CB	GLU A 807	34.424	2.804	56.557	1.00	34.12	A	ATOM 1507	CG	ASN A 813	44.853	5.042	40.086	1.00	26.28	A
ATOM 1457	CG	GLU A 807	34.550	3.974	57.511	1.00	39.14	A	ATOM 1508	OD1	ASN A 813	44.253	4.260	40.821	1.00	28.83	A
ATOM 1458	CD	GLU A 807	35.002	3.567	58.901	1.00	41.03	A	ATOM 1509	ND2	ASN A 813	44.634	5.103	38.780	1.00	30.09	A
ATOM 1459	OE1	GLU A 807	35.735	2.559	59.033	1.00	40.59	A	ATOM 1510	C	ASN A 813	44.144	7.641	41.282	1.00	22.37	A
ATOM 1460	OE2	GLU A 807	34.632	4.274	59.866	1.00	44.06	A	ATOM 1511	O	ASN A 813	44.189	8.298	40.242	1.00	23.21	A
ATOM 1461	C	GLU A 807	34.219	2.130	54.156	1.00	33.16	A	ATOM 1512	N	TYR A 814	43.056	7.567	42.038	1.00	22.02	A
ATOM 1462	O	GLU A 807	33.419	1.217	53.984	1.00	33.91	A	ATOM 1513	CA	TYR A 814	41.830	8.258	41.668	1.00	20.72	A
ATOM 1463	N	GLY A 808	35.359	2.232	53.480	1.00	32.20	A	ATOM 1514	CB	TYR A 814	40.618	7.407	42.041	1.00	21.99	A
ATOM 1464	CA	GLY A 808	35.718	1.241	52.481	1.00	30.35	A	ATOM 1515	CG	TYR A 814	40.094	6.529	40.930	1.00	23.19	A
ATOM 1465	C	GLY A 808	37.007	1.598	51.769	1.00	28.25	A	ATOM 1516	CD1	TYR A 814	40.934	5.643	40.254	1.00	23.38	A
ATOM 1466	O	GLY A 808	37.559	2.675	51.970	1.00	27.30	A	ATOM 1517	CE1	TYR A 814	40.451	4.833	39.230	1.00	23.75	A
ATOM 1467	N	TYR A 809	37.488	0.691	50.932	1.00	27.05	A	ATOM 1518	CD2	TYR A 814	38.756	6.584	40.555	1.00	23.58	A
ATOM 1468	CA	TYR A 809	38.724	0.911	50.194	1.00	25.72	A	ATOM 1519	CE2	TYR A 814	38.263	5.782	39.533	1.00	24.91	A
ATOM 1469	CB	TYR A 809	39.348	-0.428	49.803	1.00	25.82	A	ATOM 1520	CZ	TYR A 814	39.114	4.911	38.877	1.00	24.17	A
ATOM 1470	CG	TYR A 809	40.157	-1.034	50.890	1.00	27.06	A	ATOM 1521	OH	TYR A 814	38.621	4.124	37.867	1.00	26.89	A
ATOM 1471	CD1	TYR A 809	41.353	-0.531	51.344	1.00	26.80	A	ATOM 1522	C	TYR A 814	41.723	9.592	42.391	1.00	22.20	A
ATOM 1472	CE1	TYR A 809	42.144	-1.186	52.288	1.00	27.52	A	ATOM 1523	O	TYR A 814	40.740	10.320	42.215	1.00	22.61	A
ATOM 1473	CD2	TYR A 809	39.765	-2.324	51.418	1.00	28.31	A	ATOM 1524	N	PHE A 815	42.736	9.919	43.185	1.00	20.11	A
ATOM 1474	CE2	TYR A 809	40.545	-2.986	52.363	1.00	27.20	A	ATOM 1525	CA	PHE A 815	42.690	11.135	43.978	1.00	20.73	A
ATOM 1475	CZ	TYR A 809	41.731	-2.419	52.790	1.00	28.37	A	ATOM 1526	CB	PHE A 815	42.640	10.751	45.459	1.00	20.89	A
ATOM 1476	OH	TYR A 809	42.517	-3.106	53.686	1.00	30.07	A	ATOM 1527	CG	PHE A 815	41.449	9.921	45.814	1.00	20.64	A

ATOM 1528	CD1	PHE A 815	40.261	10.527	46.224	1.00	20.64	A	ATOM 1579	CG1	VAL A 821	39.091	25.059	36.390	1.00	45.68	A
ATOM 1529	CD2	PHE A 815	41.490	8.538	45.678	1.00	17.89	A	ATOM 1580	CG2	VAL A 821	40.937	24.245	37.839	1.00	44.32	A
ATOM 1530	CE1	PHE A 815	39.125	9.762	46.493	1.00	20.76	A	ATOM 1581	C	VAL A 821	37.547	22.753	36.877	1.00	48.31	A
ATOM 1531	CE2	PHE A 815	40.361	7.762	45.943	1.00	19.45	A	ATOM 1582	O	VAL A 821	37.633	22.362	35.711	1.00	49.80	A
ATOM 1532	CZ	PHE A 815	39.179	8.371	46.350	1.00	19.78	A	ATOM 1583	N	LYS A 822	36.382	23.025	37.456	1.00	51.40	A
ATOM 1533	C	PHE A 815	43.749	12.200	43.795	1.00	20.60	A	ATOM 1584	CA	LYS A 822	35.133	22.869	36.718	1.00	54.92	A
ATOM 1534	O	PHE A 815	44.891	11.932	43.416	1.00	18.47	A	ATOM 1585	CB	LYS A 822	34.128	22.048	37.533	1.00	56.04	A
ATOM 1535	N	GLY A 816	43.332	13.425	44.099	1.00	22.98	A	ATOM 1586	CG	LYS A 822	34.512	20.575	37.639	1.00	58.37	A
ATOM 1536	CA	GLY A 816	44.210	14.569	44.025	1.00	23.64	A	ATOM 1587	CD	LYS A 822	34.796	19.998	36.256	1.00	58.10	A
ATOM 1537	C	GLY A 816	44.266	15.225	45.396	1.00	25.20	A	ATOM 1588	CE	LYS A 822	35.391	18.607	36.336	1.00	58.92	A
ATOM 1538	O	GLY A 816	43.450	14.940	46.275	1.00	22.75	A	ATOM 1589	NZ	LYS A 822	35.780	18.110	34.984	1.00	58.38	A
ATOM 1539	N	LEU A 817	45.247	16.095	45.587	1.00	26.71	A	ATOM 1590	C	LYS A 822	34.514	24.197	36.316	1.00	55.73	A
ATOM 1540	CA	LEU A 817	45.386	16.807	46.842	1.00	29.12	A	ATOM 1591	O	LYS A 822	35.143	25.242	36.581	1.00	56.79	A
ATOM 1541	CB	LEU A 817	46.819	17.326	46.972	1.00	31.43	A	ATOM 1592	OXT	LYS A 822	33.412	24.170	35.728	1.00	57.20	A
ATOM 1542	CG	LEU A 817	47.405	17.656	48.346	1.00	31.74	A	ATOM 1	O	HOH W 1	46.269	17.160	43.291	1.00	14.14	W
ATOM 1543	CD1	LEU A 817	47.129	16.529	49.335	1.00	33.55	A	ATOM 2	O	HOH W 2	31.540	12.929	53.871	1.00	25.06	W
ATOM 1544	CD2	LEU A 817	48.909	17.871	48.190	1.00	30.87	A	ATOM 3	O	HOH W 3	35.797	26.743	22.574	1.00	17.35	W
ATOM 1545	C	LEU A 817	44.376	17.952	46.755	1.00	29.10	A	ATOM 4	O	HOH W 4	42.189	22.413	34.190	1.00	26.99	W
ATOM 1546	O	LEU A 817	43.869	18.432	47.767	1.00	32.47	A	ATOM 5	O	HOH W 5	44.885	19.883	23.147	1.00	21.36	W
ATOM 1547	N	PHE A 818	44.073	18.352	45.522	1.00	28.98	A	ATOM 6	O	HOH W 6	45.207	14.689	52.672	1.00	26.26	W
ATOM 1548	CA	PHE A 818	43.118	19.417	45.215	1.00	28.06	A	ATOM 7	O	HOH W 7	43.086	-8.032	48.050	1.00	29.04	W
ATOM 1549	CB	PHE A 818	43.844	20.639	44.637	1.00	28.59	A	ATOM 8	O	HOH W 8	45.160	20.520	26.791	1.00	23.45	W
ATOM 1550	CG	PHE A 818	44.689	21.368	45.630	1.00	29.54	A	ATOM 9	O	HOH W 9	29.974	-0.001	49.799	1.00	26.85	W
ATOM 1551	CD1	PHE A 818	44.222	22.526	46.241	1.00	31.72	A	ATOM 10	O	HOH W 10	41.301	18.968	34.883	1.00	27.70	W
ATOM 1552	CD2	PHE A 818	45.937	20.879	45.990	1.00	29.61	A	ATOM 11	O	HOH W 11	48.364	3.177	44.083	1.00	35.62	W
ATOM 1553	CE1	PHE A 818	44.990	23.183	47.202	1.00	30.12	A	ATOM 12	O	HOH W 12	48.786	14.897	35.533	1.00	26.37	W
ATOM 1554	CE2	PHE A 818	46.707	21.530	46.947	1.00	29.41	A	ATOM 13	O	HOH W 13	45.068	18.381	28.301	1.00	20.87	W
ATOM 1555	CZ	PHE A 818	46.232	22.683	47.553	1.00	28.12	A	ATOM 14	O	HOH W 14	37.342	13.234	58.416	1.00	26.77	W
ATOM 1556	C	PHE A 818	42.155	18.883	44.158	1.00	29.01	A	ATOM 15	O	HOH W 15	55.793	7.365	44.154	1.00	31.94	W
ATOM 1557	O	PHE A 818	42.461	17.915	43.470	1.00	28.06	A	ATOM 16	O	HOH W 16	73.449	14.689	37.176	1.00	31.98	W
ATOM 1558	N	PRO A 819	40.982	19.515	44.012	1.00	30.16	A	ATOM 17	O	HOH W 17	45.296	34.795	7.454	1.00	27.21	W
ATOM 1559	CD	PRO A 819	40.418	20.525	44.928	1.00	31.17	A	ATOM 18	O	HOH W 18	41.404	11.989	32.618	1.00	24.12	W
ATOM 1560	CA	PRO A 819	39.980	19.093	43.025	1.00	32.38	A	ATOM 19	O	HOH W 19	51.278	21.938	46.693	1.00	25.75	W
ATOM 1561	CB	PRO A 819	38.681	19.588	43.639	1.00	32.27	A	ATOM 20	O	HOH W 20	50.195	4.489	42.134	1.00	29.12	W
ATOM 1562	CG	PRO A 819	39.117	20.906	44.242	1.00	31.04	A	ATOM 21	O	HOH W 21	46.587	9.910	43.029	1.00	29.64	W
ATOM 1563	C	PRO A 819	40.220	19.713	41.641	1.00	33.44	A	ATOM 22	O	HOH W 22	38.964	14.921	59.152	1.00	27.92	W
ATOM 1564	O	PRO A 819	40.885	20.743	41.525	1.00	32.32	A	ATOM 23	O	HOH W 23	59.997	15.377	50.671	1.00	38.16	W
ATOM 1565	N	ARG A 820	39.670	19.088	40.601	1.00	33.62	A	ATOM 24	O	HOH W 24	54.398	31.040	28.054	1.00	40.59	W
ATOM 1566	CA	ARG A 820	39.809	19.602	39.238	1.00	35.29	A	ATOM 25	O	HOH W 25	53.446	27.077	40.483	1.00	29.95	W
ATOM 1567	CB	ARG A 820	39.122	18.684	38.227	1.00	31.84	A	ATOM 26	O	HOH W 26	38.014	16.961	41.007	1.00	29.57	W
ATOM 1568	CG	ARG A 820	39.725	17.306	38.031	1.00	32.86	A	ATOM 27	O	HOH W 27	54.078	10.983	42.220	1.00	23.94	W
ATOM 1569	CD	ARG A 820	38.778	16.496	37.145	1.00	30.43	A	ATOM 28	O	HOH W 28	69.713	18.131	39.304	1.00	30.88	W
ATOM 1570	NE	ARG A 820	39.269	15.167	36.814	1.00	29.35	A	ATOM 29	O	HOH W 29	30.688	1.307	56.570	1.00	29.15	W
ATOM 1571	CZ	ARG A 820	40.177	14.908	35.878	1.00	32.49	A	ATOM 30	O	HOH W 30	44.731	7.951	37.710	1.00	37.76	W
ATOM 1572	NH1	ARG A 820	40.708	15.899	35.165	1.00	32.71	A	ATOM 31	O	HOH W 31	38.480	-3.945	42.873	1.00	32.30	W
ATOM 1573	NH2	ARG A 820	40.541	13.654	35.643	1.00	29.43	A	ATOM 32	O	HOH W 32	49.302	20.196	9.313	1.00	38.33	W
ATOM 1574	C	ARG A 820	39.159	20.977	39.128	1.00	37.29	A	ATOM 33	O	HOH W 33	48.445	12.832	37.031	1.00	29.79	W
ATOM 1575	O	ARG A 820	38.471	21.425	40.037	1.00	38.81	A	ATOM 34	O	HOH W 34	51.626	27.011	15.089	1.00	26.93	W
ATOM 1576	N	VAL A 821	39.373	21.630	37.993	1.00	41.05	A	ATOM 35	O	HOH W 35	47.229	-3.126	46.545	1.00	35.96	W
ATOM 1577	CA	VAL A 821	38.804	22.943	37.724	1.00	44.92	A	ATOM 36	O	HOH W 36	63.659	8.989	38.540	1.00	34.36	W
ATOM 1578	CB	VAL A 821	39.798	23.830	36.938	1.00	44.42	A	ATOM 37	O	HOH W 37	41.784	21.059	36.559	1.00	31.44	W

ATOM	38	O	HOH	W	38	66.756	23.200	41.632	1.00	36.15	W	ATOM	89	O	HOH	W	89	53.020	3.253	42.614	1.00	39.52	W
ATOM	39	O	HOH	W	39	67.912	13.992	30.290	1.00	32.00	W	ATOM	90	O	HOH	W	90	62.965	22.337	34.911	1.00	45.12	W
ATOM	40	O	HOH	W	40	49.207	29.450	8.808	1.00	28.53	W	ATOM	91	O	HOH	W	91	28.847	9.829	46.687	1.00	35.84	W
ATOM	41	O	HOH	W	41	37.344	18.760	52.185	1.00	32.55	W	ATOM	92	O	HOH	W	92	62.642	7.179	42.244	1.00	46.08	W
ATOM	42	O	HOH	W	42	52.711	24.798	13.345	1.00	36.95	W	ATOM	93	O	HOH	W	93	30.058	15.860	48.986	1.00	68.81	W
ATOM	43	O	HOH	W	43	36.662	-7.658	48.925	1.00	38.53	W	ATOM	94	O	HOH	W	94	34.480	-11.246	47.129	1.00	38.88	W
ATOM	44	O	HOH	W	44	39.374	19.610	33.206	1.00	30.70	W	ATOM	95	O	HOH	W	95	72.315	17.937	35.140	1.00	34.08	W
ATOM	45	O	HOH	W	45	52.379	19.611	16.208	1.00	38.12	W	ATOM	96	O	HOH	W	96	51.149	4.098	51.727	1.00	38.76	W
ATOM	46	O	HOH	W	46	29.136	7.518	51.070	1.00	32.79	W	ATOM	97	O	HOH	W	97	38.379	8.473	32.402	1.00	41.76	W
ATOM	47	O	HOH	W	47	48.856	7.517	33.878	1.00	27.81	W	ATOM	98	O	HOH	W	98	58.068	5.623	44.978	1.00	44.18	W
ATOM	48	O	HOH	W	48	50.307	28.509	13.585	1.00	33.49	W	ATOM	99	O	HOH	W	99	56.805	30.754	27.064	1.00	52.94	W
ATOM	49	O	HOH	W	49	69.226	20.627	32.602	1.00	51.51	W	ATOM	100	O	HOH	W	100	29.363	1.253	36.994	1.00	63.86	W
ATOM	50	O	HOH	W	50	41.843	30.680	30.582	1.00	40.55	W	ATOM	101	O	HOH	W	101	65.980	9.221	40.622	1.00	39.08	W
ATOM	51	O	HOH	W	51	69.590	21.839	40.002	1.00	35.62	W	ATOM	102	O	HOH	W	102	42.846	20.954	57.938	1.00	34.33	W
ATOM	52	O	HOH	W	52	34.708	9.151	35.581	1.00	40.37	W	ATOM	103	O	HOH	W	103	53.044	27.285	35.683	1.00	43.54	W
ATOM	53	O	HOH	W	53	75.474	7.527	34.776	1.00	32.52	W	ATOM	104	O	HOH	W	104	48.062	32.940	30.875	1.00	36.29	W
ATOM	54	O	HOH	W	54	35.928	25.337	39.194	1.00	53.85	W	ATOM	105	O	HOH	W	105	42.332	-6.342	51.086	1.00	36.84	W
ATOM	55	O	HOH	W	55	38.922	-6.027	39.916	1.00	33.00	W	ATOM	106	O	HOH	W	106	36.480	14.292	53.861	1.00	42.84	W
ATOM	56	O	HOH	W	56	43.013	39.184	19.941	1.00	39.13	W	ATOM	107	O	HOH	W	107	37.038	23.027	41.503	1.00	45.04	W
ATOM	57	O	HOH	W	57	30.959	-7.723	47.845	1.00	26.55	W	ATOM	108	O	HOH	W	108	44.157	16.639	13.766	1.00	33.85	W
ATOM	58	O	HOH	W	58	31.432	25.013	19.231	1.00	38.63	W	ATOM	109	O	HOH	W	109	65.932	10.121	34.336	1.00	46.09	W
ATOM	59	O	HOH	W	59	33.742	28.174	19.452	1.00	40.19	W	ATOM	110	O	HOH	W	110	62.444	10.291	33.720	1.00	34.42	W
ATOM	60	O	HOH	W	60	45.981	3.159	42.700	1.00	36.90	W	ATOM	111	O	HOH	W	111	64.195	10.401	44.231	1.00	41.24	W
ATOM	61	O	HOH	W	61	50.334	8.823	51.516	1.00	35.93	W	ATOM	112	O	HOH	W	112	47.595	6.307	57.773	1.00	47.70	W
ATOM	62	O	HOH	W	62	43.720	18.121	50.311	1.00	34.65	W	ATOM	113	O	HOH	W	113	46.020	4.538	18.362	1.00	52.45	W
ATOM	63	O	HOH	W	63	62.681	23.049	38.768	1.00	46.28	W	ATOM	114	O	HOH	W	114	49.951	6.009	50.447	1.00	44.83	W
ATOM	64	O	HOH	W	64	47.167	1.012	52.172	1.00	29.55	W	ATOM	115	O	HOH	W	115	31.016	27.219	20.748	1.00	44.20	W
ATOM	65	O	HOH	W	65	37.882	24.734	24.133	1.00	45.56	W	ATOM	116	O	HOH	W	116	32.435	30.309	19.435	1.00	53.93	W
ATOM	66	O	HOH	W	66	33.427	-7.241	51.112	1.00	33.75	W	ATOM	117	O	HOH	W	117	45.066	-7.910	51.150	1.00	43.51	W
ATOM	67	O	HOH	W	67	34.201	12.697	53.106	1.00	36.55	W	ATOM	118	O	HOH	W	118	47.201	5.052	36.509	1.00	38.90	W
ATOM	68	O	HOH	W	68	31.268	16.175	43.431	1.00	45.89	W	ATOM	119	O	HOH	W	119	34.009	25.891	20.234	1.00	53.50	W
ATOM	69	O	HOH	W	69	41.312	2.677	60.643	1.00	48.86	W	ATOM	120	O	HOH	W	120	36.332	27.310	37.462	1.00	58.09	W
ATOM	70	O	HOH	W	70	42.131	14.747	26.495	1.00	33.12	W	ATOM	121	O	HOH	W	121	62.999	6.700	39.516	1.00	50.67	W
ATOM	71	O	HOH	W	71	34.146	2.435	38.498	1.00	36.69	W	ATOM	122	O	HOH	W	122	56.242	9.452	26.457	1.00	36.07	W
ATOM	72	O	HOH	W	72	45.557	8.407	57.158	1.00	36.76	W	ATOM	123	O	HOH	W	123	52.408	9.569	23.353	1.00	44.65	W
ATOM	73	O	HOH	W	73	34.635	-0.500	39.353	1.00	47.35	W	ATOM	124	O	HOH	W	124	51.437	19.989	48.928	1.00	49.65	W
ATOM	74	O	HOH	W	74	36.600	25.272	33.263	1.00	52.78	W	ATOM	125	O	HOH	W	125	56.807	31.564	30.051	1.00	50.01	W
ATOM	75	O	HOH	W	75	28.255	11.620	38.182	1.00	45.94	W	ATOM	126	O	HOH	W	126	56.691	20.979	16.707	1.00	50.74	W
ATOM	76	O	HOH	W	76	44.102	40.958	16.741	1.00	44.75	W	ATOM	127	O	HOH	W	127	38.465	29.953	26.555	1.00	40.37	W
ATOM	77	O	HOH	W	77	62.525	9.763	41.508	1.00	32.70	W	ATOM	128	O	HOH	W	128	31.913	18.730	42.892	1.00	43.47	W
ATOM	78	O	HOH	W	78	55.241	2.819	40.705	1.00	29.67	W	ATOM	129	O	HOH	W	129	60.835	15.134	28.976	1.00	51.05	W
ATOM	79	O	HOH	W	79	46.132	-7.956	57.187	1.00	43.11	W	ATOM	130	O	HOH	W	130	50.410	7.832	15.656	1.00	53.72	W
ATOM	80	O	HOH	W	80	42.130	23.013	41.996	1.00	31.74	W	ATOM	131	O	HOH	W	131	37.771	2.061	60.561	1.00	59.75	W
ATOM	81	O	HOH	W	81	26.129	6.867	53.914	1.00	51.56	W	ATOM	132	O	HOH	W	132	47.821	-3.854	43.832	1.00	37.10	W
ATOM	82	O	HOH	W	82	44.791	9.699	33.485	1.00	43.62	W	ATOM	133	O	HOH	W	133	53.112	0.770	43.851	1.00	42.48	W
ATOM	83	O	HOH	W	83	54.675	4.926	44.623	1.00	35.27	W	ATOM	134	O	HOH	W	134	42.129	-5.647	53.823	1.00	48.54	W
ATOM	84	O	HOH	W	84	52.605	25.839	10.677	1.00	36.60	W	ATOM	135	O	HOH	W	135	52.254	22.724	10.344	1.00	47.22	W
ATOM	85	O	HOH	W	85	30.057	-1.593	47.775	1.00	46.57	W	ATOM	136	O	HOH	W	136	37.340	-11.127	46.280	1.00	39.82	W
ATOM	86	O	HOH	W	86	64.560	20.180	32.259	1.00	61.78	W	ATOM	137	O	HOH	W	137	39.934	14.257	28.197	1.00	38.05	W
ATOM	87	O	HOH	W	87	45.565	12.312	26.336	1.00	34.43	W	ATOM	138	O	HOH	W	138	53.766	34.310	22.955	1.00	58.01	W
ATOM	88	O	HOH	W	88	42.473	6.886	31.180	1.00	45.33	W	ATOM	139	O	HOH	W	139	45.438	13.434	59.731	1.00	48.69	W

ATOM	140	O	HOH	W	140	35.839	4.027	37.082	1.00	46.65	W
ATOM	141	O	HOH	W	141	55.295	16.527	24.998	1.00	53.43	W
ATOM	142	O	HOH	W	142	43.551	29.066	41.077	1.00	49.51	W
ATOM	143	O	HOH	W	143	33.848	-8.099	41.220	1.00	39.55	W
ATOM	144	O	HOH	W	144	70.638	24.288	39.051	1.00	48.87	W
ATOM	145	O	HOH	W	145	44.681	7.258	18.628	1.00	59.53	W
ATOM	146	O	HOH	W	146	53.929	19.445	18.352	1.00	39.32	W
ATOM	147	O	HOH	W	147	32.807	21.065	25.734	1.00	56.74	W
ATOM	148	O	HOH	W	148	44.844	9.217	20.709	1.00	46.92	W
ATOM	149	O	HOH	W	149	59.576	22.445	31.821	1.00	40.14	W
ATOM	150	O	HOH	W	150	51.991	10.138	39.109	1.00	13.41	W
ATOM	151	O	HOH	W	151	64.217	24.444	41.110	1.00	60.59	W
ATOM	152	O	HOH	W	152	54.840	35.991	18.856	1.00	41.03	W
ATOM	153	O	HOH	W	153	54.039	2.999	46.764	1.00	50.27	W
ATOM	154	O	HOH	W	154	33.271	26.250	34.000	1.00	36.55	W

END

What is claimed is:

1. An isolated compound having a spatial arrangement of atoms to bind to iASPP in a manner that interferes with iASPP binding to an iASPP binding partner selected from the group consisting of p53, p63 and p73,

wherein the compound interacts with an iASPP polypeptide defined by at least 10 structural coordinates identified in Table 1, wherein the relative atomic positions of the structure identified in Table 1 are varied within a root mean square deviation of less than 1.2Å.

2. The isolated compound of claim 1, wherein the compound interacts with the iASPP in a region of iASPP defined by at least 10 coordinates of atoms from at least five different amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2Å.

3. The isolated compound of claim 1, wherein the compound interacts with the iASPP in a region of iASPP defined by at least 10 coordinates of atoms from at least 10 different amino acid residues identified in Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2Å.

4. The isolated compound of claim 3, wherein the at least 10 amino acids of iASPP are selected from the group consisting of T722, L724, W767, E772, D775, E776, E795, D797, W798, Y809, P811, N813 and Y814.

5. The isolated compound of claim 1, wherein the compound interacts with the iASPP in a region of iASPP defined by at least 20 coordinates of atoms from at least ten different amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2Å.

6. The isolated compound of claim 1, wherein the compound interacts with the iASPP in a region of iASPP defined by at least 50 coordinates of atoms from at least ten different amino acids of Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2Å.

7. An isolated compound of any one of claims 1-6 having a structure selected from the group consisting of:

(a) a compound that comprises a sequence of eight amino acids X1-X2-X3-X4-X5-X6-X7-X8, wherein X1 is selected from Ser or Thr; X2 is Pro; X3, X4, X6, and X7 are each independently selected from Arg and Lys; X5 is Ala, Ser, Val, or Gly; and X8 is Ala, Val, or Gly;

(b) a compound with the structure of (a), with the proviso that one or more of X1-X2-X3-X4-X5-X6-X7-X8 amino acids are replaced with an unnatural amino acid; and

(c) a compound with the structure of (a) or (b), with the proviso that one or more amide bonds is replaced with an ester or alkyl bond.

8. An isolated compound of claim 7 that comprises a sequence of eight amino acids X1-X2-X3-X4-X5-X6-X7-X8, wherein X1 is selected from Ser or Thr; X2 is Pro; X3, X4, X6, and X7 are each independently selected from Arg and Lys; X5 is Ala, Ser, Val, or Gly; and X8 is Ala, Val, or Gly.

9. The isolated compound of any one of claims 1-6, wherein the compound is selected from the group consisting of a small molecule, an antibody or an antigen-binding fragment thereof, a nucleic acid molecule, and a polypeptide.

10. The isolated compound of claim 9, wherein the compound is a polypeptide.

11. The isolated compound of claim 10, wherein the polypeptide comprises an amino acid sequence at least 80% identical to SEQ ID NO: 2.

12. The isolated compound of claim 9, wherein the compound is a nucleic acid.

13. The isolated compound of claim 9, wherein the compound is an antibody or an antigen binding fragment thereof.

14. The isolated compound of claim 13, wherein the antibody or antigen binding fragment thereof specifically binds to an iASPP epitope defined by at least 10 coordinates of atoms from at least 10 different amino acid residues identified in Table 3, wherein the relative atomic positions are varied within a root mean square deviation of less than 1.2Å.

15. The isolated compound of claim 13, wherein the antibody or antigen binding fragment thereof specifically binds to an epitope that includes one or more iASPP amino acids selected from the group consisting of T722, L724, W767, E772, D775, E776, E795, D797, W798, Y809, P811, N813 and Y814.

16. The isolated compound of any one of claims 13-15, wherein the antibody is a monoclonal antibody.

17. The isolated compound of claim 16, wherein the antibody is a human antibody or a humanized antibody.

18. A hybridoma cell that produces the antibody of claim 16 or 17.

19. An isolated compound that comprises a sequence of eight amino acids X1-X2-X3-X4-X6-X7-X8, wherein

X1 is selected from Ser or Thr;

X2 is Pro;

X3, X4, X6, and X7 are each independently selected from Arg and Lys;

X5 is Ala, Ser, Val, or Gly; and

X8 is Ala, Val, or Gly;

wherein the compound inhibits binding between iASPP and an iASPP binding partner selected from the group consisting of p53, p63, and p73.

20. The compound of claim 19 that comprises an amino acid sequence at least 80% identical to iASPP amino acids 616-623 (SEQ ID NO: 2).

21. The compound of claim 19 that comprises iASPP amino acids 616-623 (SEQ ID NO: 2).

22. The isolated compound of claim 20, wherein the polypeptide comprises the amino acid sequence SPRKSRRA (SEQ ID NO: 3).

23. The isolated compound of claim 20, wherein the polypeptide comprises the amino acid sequence SPRRARRA (SEQ ID NO: 4).

24. The isolated compound of claim 20, wherein the polypeptide comprises the amino acid sequence SPRRSRRA (SEQ ID NO: 5).
25. The isolated compound of any one of claims 1-24, wherein the compound further comprises at least one attached polyalkyleneglycol moiety.
26. The isolated compound of any one of claims 1-25, wherein the compound further includes at least one esterified acid side chain.
27. The isolated compound of any one of claims 1-26, wherein the compound is cyclized.
28. The isolated compound of any one of claims 1-27, wherein the compound further comprises an attached cytotoxic moiety.
29. The isolated compound of any one of claims 1-28, wherein the compound comprises a peptide.
30. A composition comprising the compound of any one of claims 1-29, and a pharmaceutically acceptable carrier, diluent or excipient.
31. A nucleic acid molecule comprising a nucleotide sequence that encodes the isolated peptide of claim 29.
32. The nucleic acid of claim 31, further comprising a promoter that promotes expression of the nucleotide sequence in cells of the mammalian subject.
33. A vector comprising the nucleic acid of claim 31 or 32.
34. The vector of claim 33, selected from the group consisting of a replication deficient retrovirus, adenovirus, adeno-associated virus, or lentivirus vector.
35. A host cell transformed or transfected with a nucleic acid or vector according to any one of claims 31-34
36. A method of treating a neoplastic disorder comprising:
administering to a subject in need of treatment for a neoplastic disorder the composition of claim 30, in an amount effective to treat the neoplastic disorder.

37. The method of claim 36 further comprising a step, prior to the administering step, of identifying a subject with a neoplastic disorder.
38. The method of claim 37, wherein the identifying step comprises identifying a subject with a neoplastic disorder characterized by elevated expression of an iASPP polypeptide in the neoplastic cells.
39. The method of claim 38, wherein the identifying step comprises screening a biological sample suspected of containing neoplastic cells from the subject for elevated expression of the iASPP polypeptide.
40. The method of claim 39, wherein the screening comprises comparing expression of iASPP in cells from the sample with expression of iASPP from healthy cells of the same type as the cells in the sample, wherein increased expression of the iASPP in cells from the sample compared to the expression of the iASPP in cells from the healthy cells indicates elevated expression of iASPP.
41. The method of any one of claims 36-40, wherein the neoplastic disorder is a cancer selected from the group consisting of breast cancer, pancreatic cancer, ovarian cancer, colorectal cancer, esophageal cancer, lung cancer, head cancer, neck cancer, gastric cancer and epithelial cancer.
42. The method of any one of claims 36-40, further comprising administering to the mammalian subject a standard of care antineoplastic therapy.
43. The method of claim 42, wherein the antineoplastic therapy is selected from the group consisting of a standard of care chemotherapeutic, a standard of care radiotherapeutic, or a standard of care radiation regimen for the neoplastic disorder.
44. A method of stimulating p53-mediated apoptosis in a cell, comprising contacting the cell with the composition according to claim 30 in an amount effective to stimulate p53-mediated apoptosis in the cell.
45. The method of claim 44, wherein the cell expresses wild type p53.
46. A method of selecting a therapeutic regimen for a subject comprising

(a) identifying a subject as having neoplastic disorder characterized by increased expression of an iASPP polypeptide; and

(b) administering to the subject the composition according to claim 30.

47. The method of claim 46, wherein the identifying step comprises screening a biological sample from the subject for increased expression of the iASPP polypeptide.

48. The method of claim 47, wherein the screening comprises comparing expression of iASPP in cells from the sample with expression of iASPP from healthy cells of the same type as the cells in the sample, wherein increased expression of the iASPP in cells from the sample compared to the expression of the iASPP in cells from the healthy cells indicates that the cell is characterized by increased expression of iASPP.

49. A method of treating a condition associated with tumor cell growth in a subject comprising administering to said subject a therapeutically effective amount of a combination therapy comprising agents selected from the group consisting of:

(a) a composition according to claim 30; and

(b) a standard of care anti-neoplastic therapy.

50. The method of claim 49, wherein the antineoplastic therapy is selected from the group consisting of a standard of care chemotherapeutic, a standard of care radiotherapeutic, or a standard of care radiation regimen for the neoplastic disorder.

51. The method of claim 49, wherein the agent(s) are administered in amounts effective to inhibit neoplastic cell growth in the subject.

52. A method of screening for a modulator of binding of iASPP with an iASPP binding partner, comprising:

(a) contacting an iASPP polypeptide comprising a structure defined by the structural coordinates provided in Table 1 with an iASPP binding partner, in the presence and absence of a test compound, under conditions in which the binding partner binds with the iASPP polypeptide;

wherein the binding partner comprises a member selected from the group consisting of p53, p63 and p73;

(b) comparing binding between said iASPP polypeptide and the binding partner in the presence and absence of the test compound, wherein increased binding identifies the test

compound as an agonist of binding, and decreased binding identifies the test compound as an antagonist of binding.

53. A computer-based method for the analysis of the interaction of a molecular structure with an iASPP structure, the method comprising

(a) fitting a molecular structure to an iASPP structure defined by the structural coordinates identified in Table 1 or selected coordinates thereof, wherein the iASPP structure is optionally varied by a root square mean deviation of not more than 1.2Å, and

(b) determining at least one interaction between an atom of said iASPP structure and said molecular structure.

54. A computer-based method of rational drug design comprising:

(a) fitting structure of at least two molecular fragments to an iASPP structure defined by the structural coordinates identified in Table 1 or selected coordinates thereof, wherein the iASPP structure is optionally varied by a root square mean deviation of not more than 1.2Å, and

(b) assembling the fitted molecular structure fragments into a single molecule to form a single molecular structure.

55. The method of claim 53 or 54, wherein said selected coordinates of the iASPP structure include atoms from one or more of the amino acid residues identified in Table 3.

56. The method of any one of claims 53-55, wherein the selected coordinates comprise at least 5 atoms.

57. The method of any one of claims 53-56, wherein said molecular structure comprises at least two atoms that are located in the same relative spatial orientation to each other and are of the same elements as a corresponding number of atoms found in any of the amino acids identified in Table 4.

58. The method of claim 57, wherein the atoms are selected from the atoms identified in Table 5.

59. The method of claim 58, wherein said atoms of Table 5 include at least two atoms that contact iASPP residues via hydrogen bonds.

60. The method of any one of claims 53-59, wherein the molecular structure is in the form of a pharmacophore.

61. The method of any one of claims 53-60, wherein the iASPP structure is a model constructed from all or a portion of the coordinates of Table 1, optionally varied by a root mean square deviation of not more than 0.5Å.

62. The method of claim 61, wherein the model is selected from the group consisting of a wire-frame model, a chicken-wire model, a ball-and-stick model, a space-filling model, a stick-model, a ribbon model, a snake model, an arrow and cylinder model, an electron density map and a molecular surface model.

63. The method of any one of claims 53-62, further comprising modifying the molecular structure to modulate its interaction with the iASPP structure.

64. A computer system, intended to generate structures and/or perform optimization of compounds that interact with an iASPP C-terminal region, the system containing computer-readable data comprising the atomic coordinate data identified in Table 1 or selected coordinates thereof, optionally varied by a root mean square deviation of not more than 1.2Å.

65. The computer system of claim 64, wherein said atomic coordinate data comprises at least one of the atoms provided by the amino acid residues identified in Tables 3 or 4.

66. The computer system of claim 64 or 65 comprising:

(a) a computer-readable data storage medium comprising data storage material encoded with said computer-readable data;

(b) a working memory for storing instructions for processing said computer-readable data; and

(c) a central-processing unit coupled to said working memory and to said computer-readable storage medium for processing said computer-readable data.

67. The computer system of claim 66, further comprising a display coupled to said central-processing unit.

68. A method for providing data for generating structures and/or performing optimization of compounds which interact with an iASPP C-terminal region, the method comprising

(a) establishing communication with a remote device containing computer-readable data comprising atomic coordinate data identified in Table 1 or selected coordinate thereof, optionally varied by a root mean square deviation of not more than 1.2Å, and

(b) receiving said computer readable data from said remote device.

69. The method of claim 68, further comprising processing said computer-readable data to display a model of an iASPP C-terminal region.

70. A computer-readable storage medium comprising a data storage material encoded with computer-readable data, wherein the data are defined by the structure identified in Table 1, optionally varied by a root mean square deviation of not more than 1.2Å.

71. A computer-readable storage medium comprising a data storage material encoded with a first set of computer-readable data comprising a Fourier transform of at least a portion of the structural coordinates for the iASPP C-terminal protein defined by the structure identified in Table 1, or selected coordinates thereof, optionally varied by a root mean square deviation of not more than 1.2Å; which data, when combined with a second set of machine readable data comprising an X-ray diffraction pattern of a molecule or molecular complex or unknown structure, using a matching programmed with the instructions for using said first set of data and said second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data.

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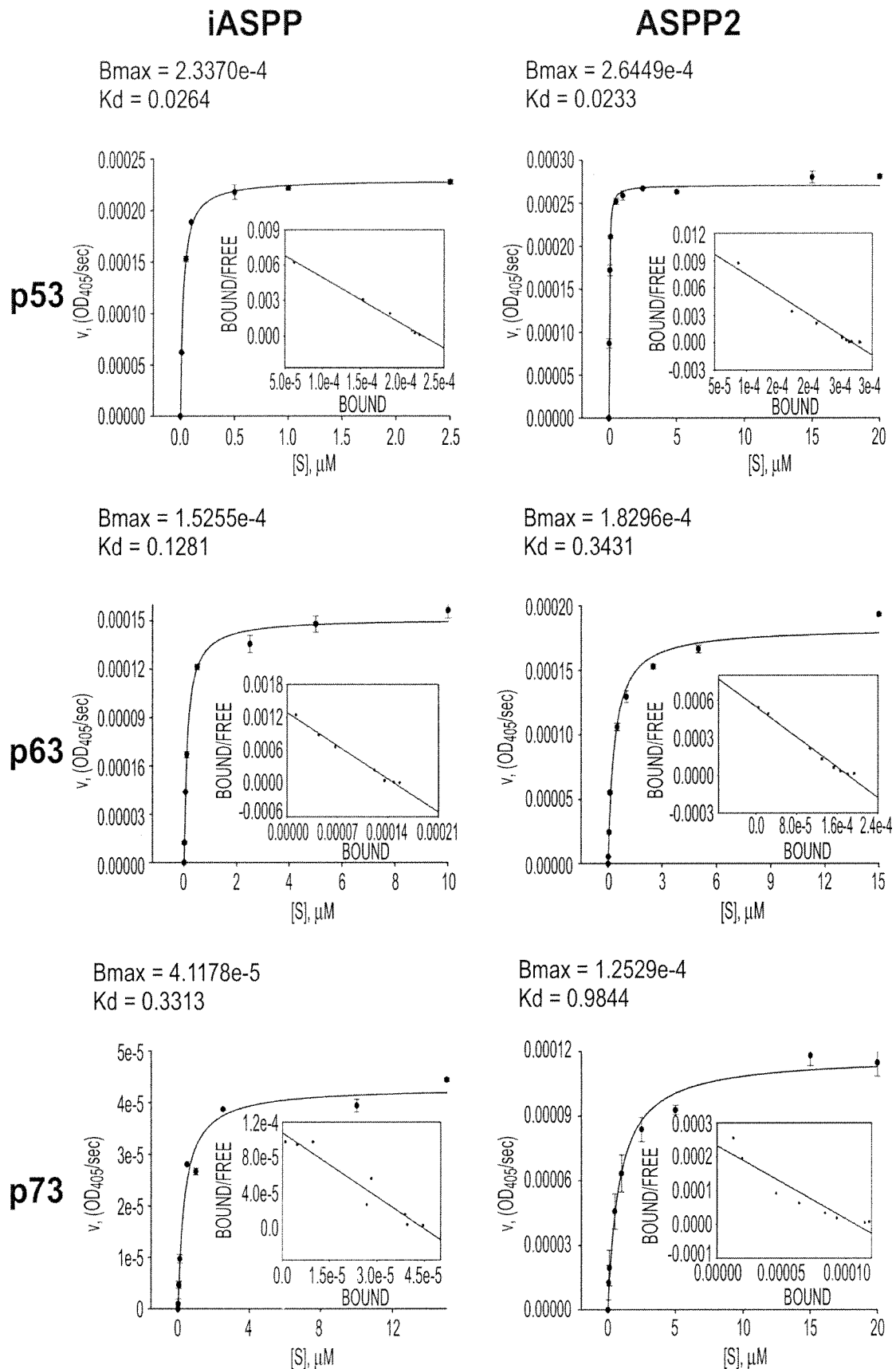
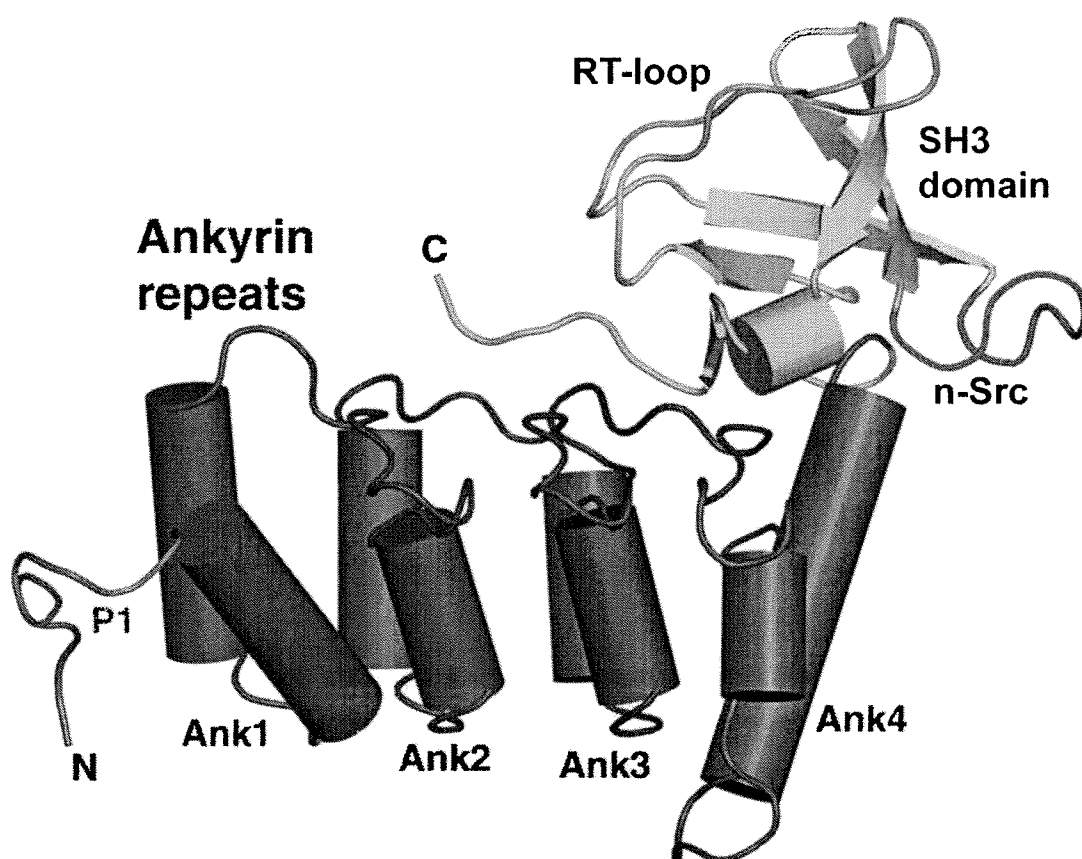


FIG. 1

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**FIG. 2A**

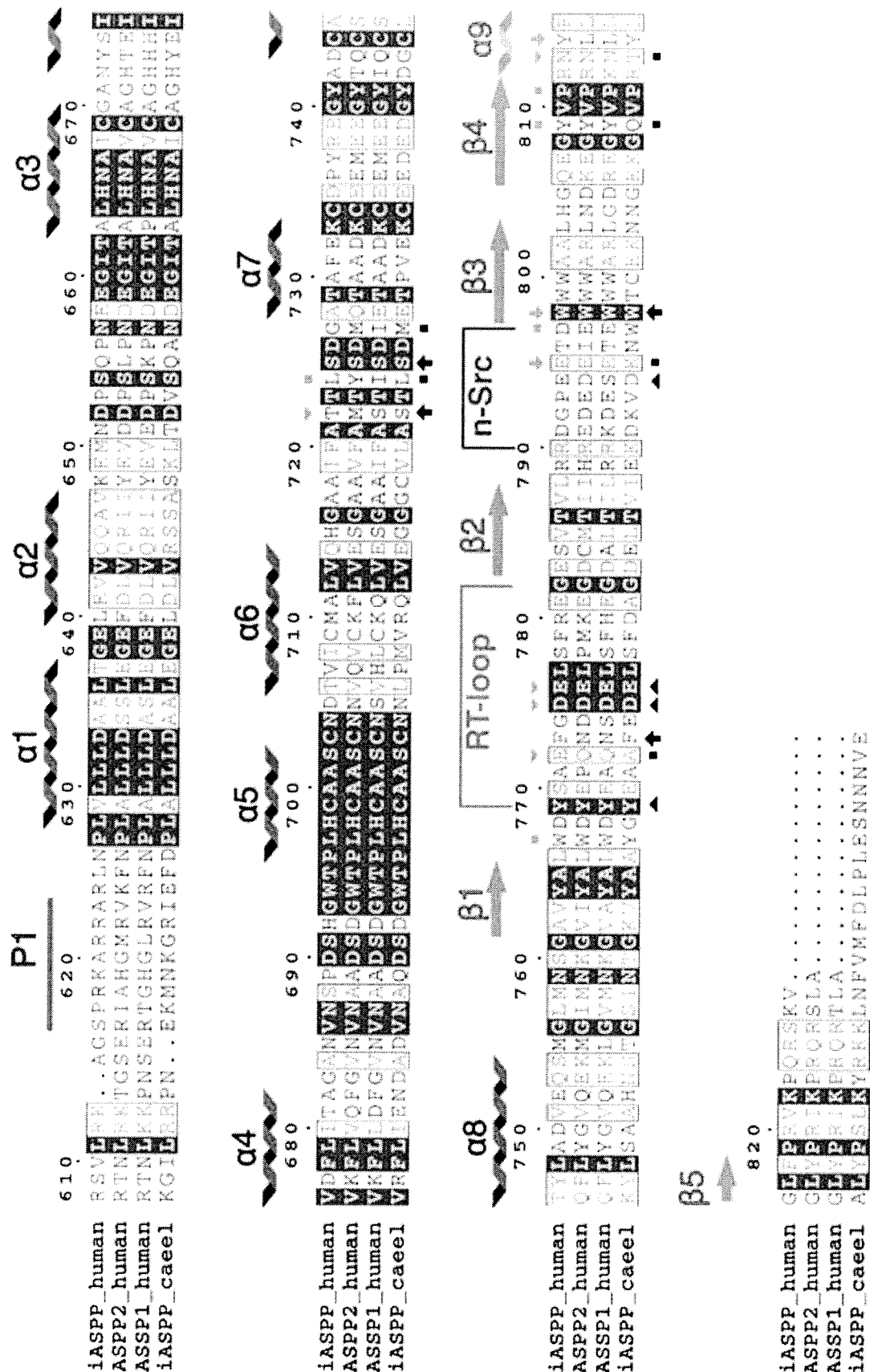
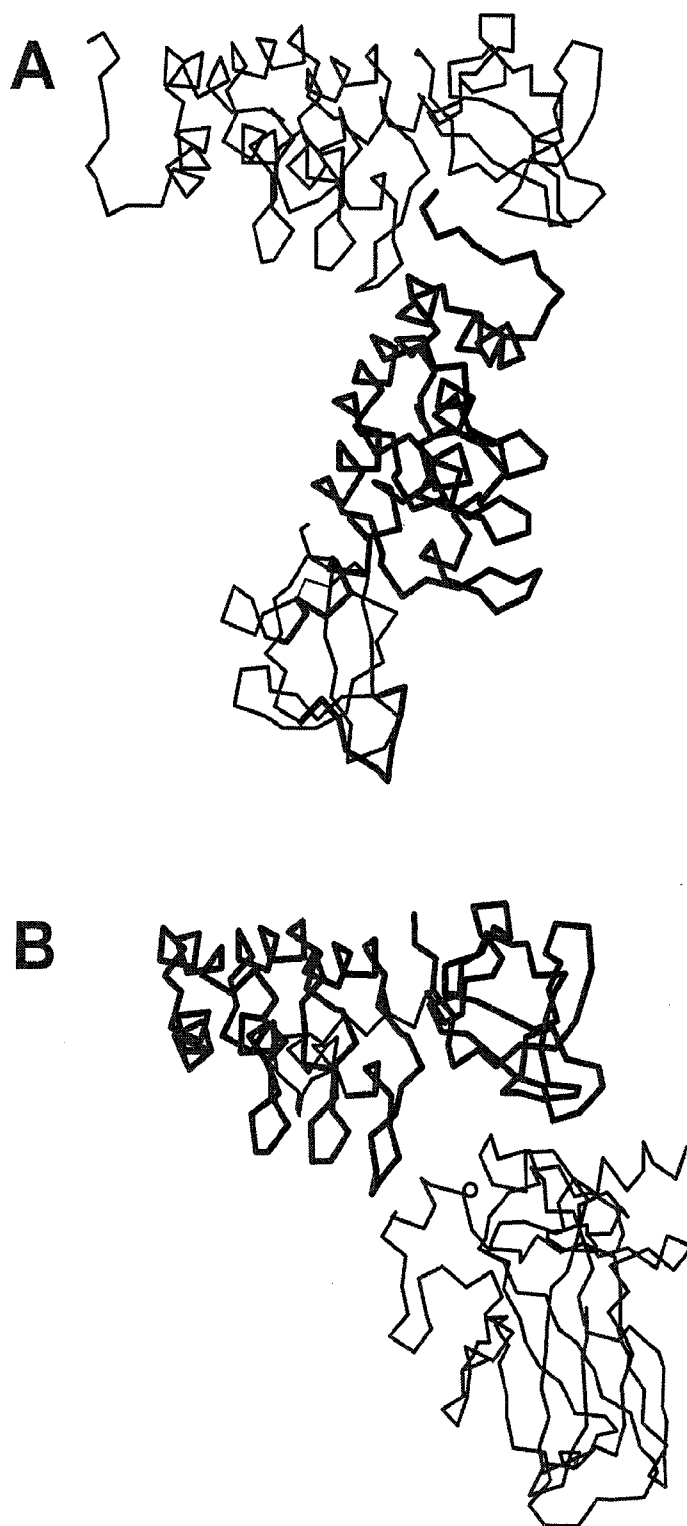
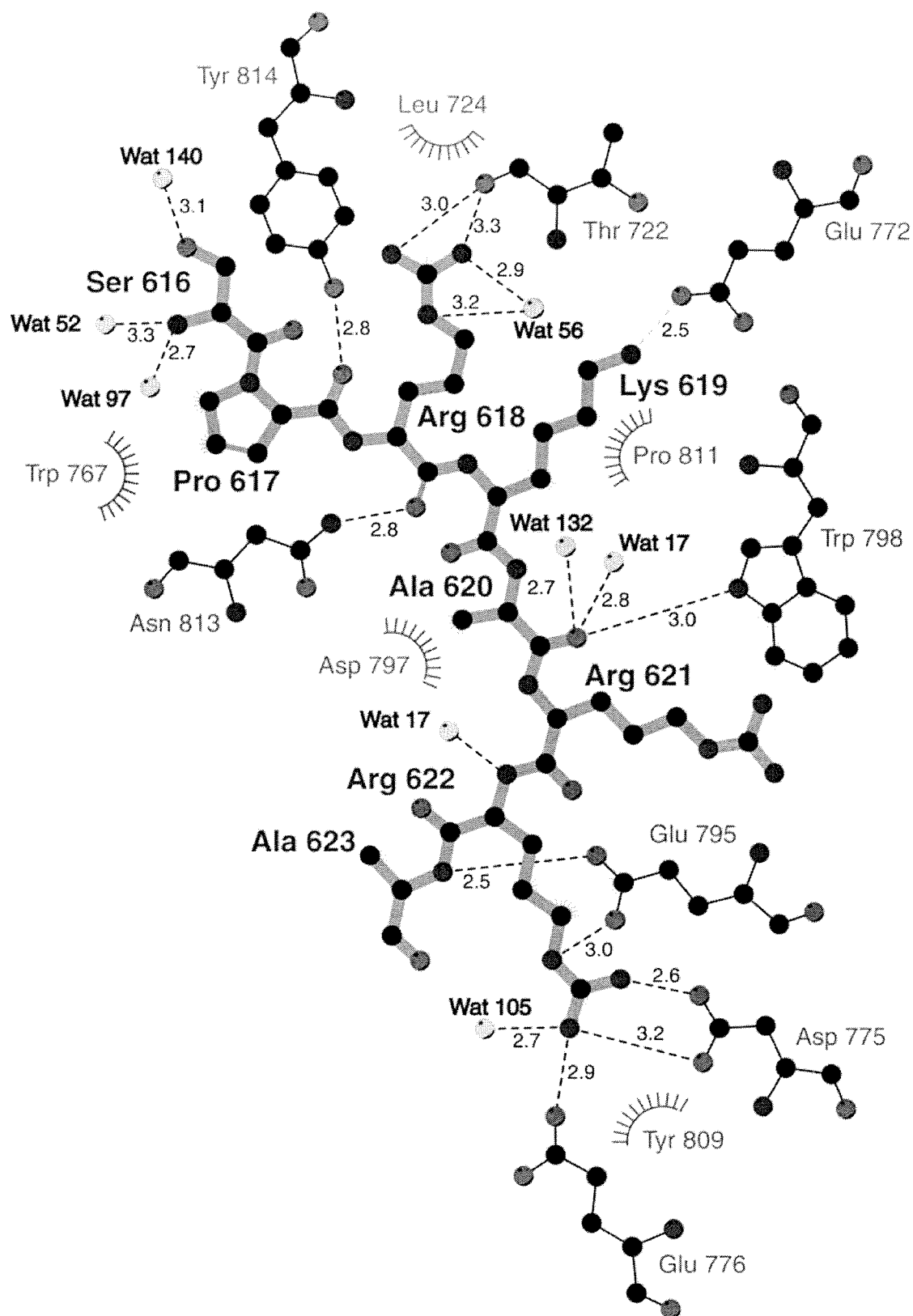


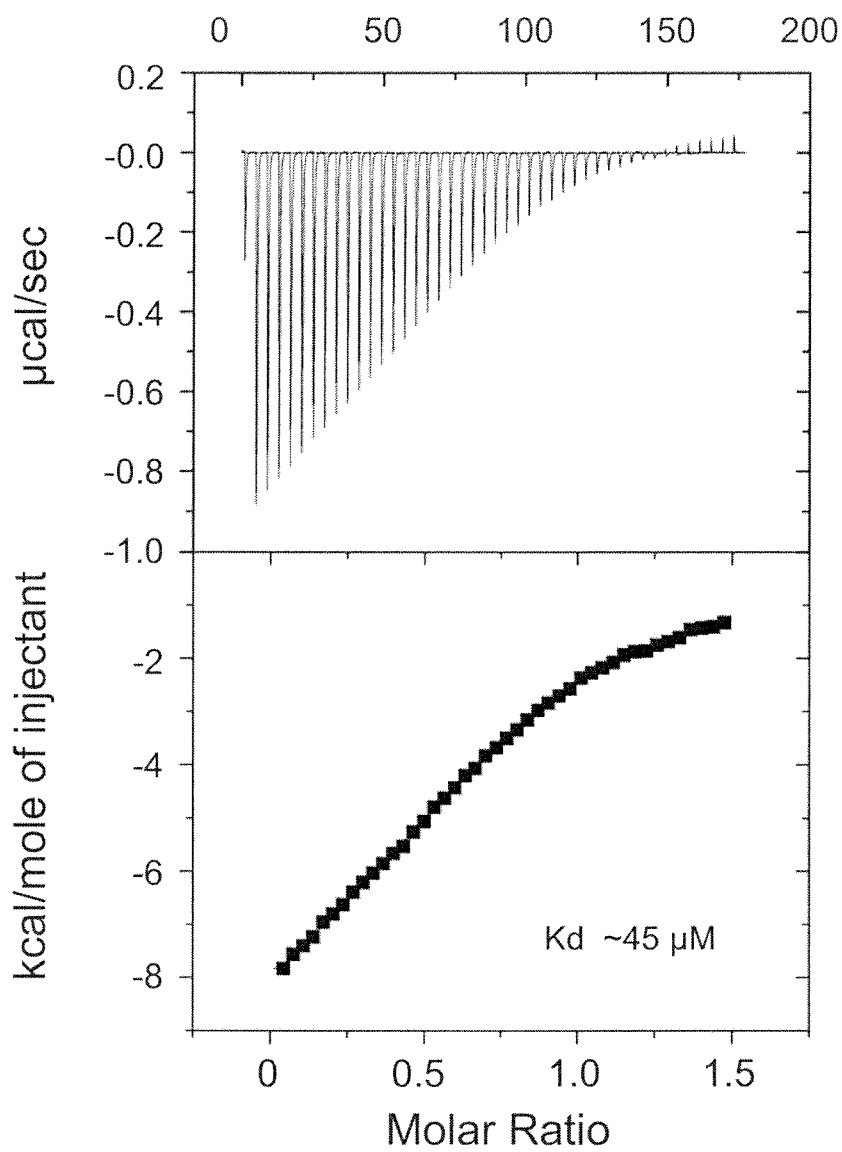
FIG. 2B

**FIG. 3**

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**FIG. 4**

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**FIG. 5**