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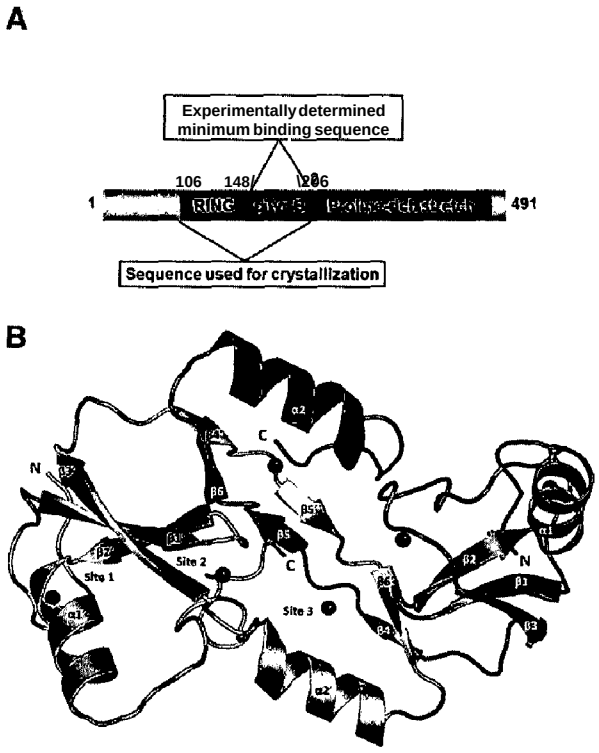

(57) Abstract: The invention concerns at least one isolated novel phosphotyrosine-binding structure typically, but not exclusively, found in Hakai protein, termed herein the *Hakai pTyr-binding* (HYB) domain, and its use in a screening assay to identify drugs for treating diseases or conditions characterised by migration or metastasis or invasion or a lack of cell-cell contact, such as cancer.

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A NOVEL PHOSPHOTYROSINE-BINDING STRUCTURE

The invention relates to at least one isolated novel phosphotyrosine-binding structure typically, but not exclusively, found in Hakai protein, termed herein the Hakai pTyr-binding (HYB) domain, and its use in a screening assay to identify drugs for treating diseases or conditions characterised by migration or metastasis or invasion or a lack of cell-cell contact, such as cancer.

Background of Invention

In eukaryotic cells, phosphorylation events regulate cell signalling by providing docking sites for protein domains, such as the Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains. The SH2 was the first signalling domain to be identified and has been extensively characterized. The SH2 is a dedicated phosphotyrosine-binding domain and plays a critical role in signal transduction, hence making it a target for drug development. Binding specificity of SH2 domains is generally conferred by the sequences flanking the C-terminus of the phosphotyrosine (pTyr), and motif recognition is usually relatively inflexible. The other major class of pTyr-binding domain is the PTB domain. The specificity of binding to the PTB domain is conferred typically by residues on the target that are N-terminal to the pTyr. However, the PTB domain also recognizes non-pTyr motifs. Atypical phosphotyrosine-binding domains have also been detected in PKCd and the human M2 pyruvate kinase (PKM2).

In 2002, Fujita et al (2002) discovered a novel ubiquitin E3 ligase protein that targeted pTyr sites on E-cadherin. The E3 ligase, Hakai protein, possesses three domains: a RING domain, a short pTyr recognition sequence and a proline-rich domain (Fujita et al, 2002). Hakai is involved in the regulation of cell adhesion, cell migration and embryogenesis (Figueroa et al, 2009; Kaido et al, 2009; Gong et al, 2010). Among the reported protein interactions of Hakai, its association with and ubiquitination of E-cadherin upon Src activation is the best characterized (Fujita et al, 2002). Based on molecular modelling, Fujita et al (2002) assumed the pTyr-binding

domain of Hakai to be a derivative SH2 domain.

In this study, we report that the Hakai pTyr-binding (HYB) domain consists of a homodimer formed at a structurally novel interface. Each monomer consists of two zinc-finger domains: a RING domain and a minimum pTyr-binding domain that incorporates a novel, atypical zinc coordination motif. Both domains play key roles in dimerization. The HYB domain is therefore composed of four zinc-binding domains co-operating to bind pTyr residues surrounded by acidic amino acids. Whereas the RING domain appears in other proteins, the atypical zinc-binding domain component is a novel protein fold that incorporates an intertwined configuration. In order to obtain its consensus target sequence, we have characterized the recognition motif of the HYB domain and identified several Src substrates that are also targeted. In addition, we have shown the HYB domain can also be found in a testis-specific ubiquitin E3 ligase, ZNF645, and the Ligand-of-Numb protein X 1 and 2 (LNX1 and LNX2).

Given the biological role of Hakai, the novel structural features of the HYB domain and its infrequent distribution among proteins the HYB domain represents a highly suitable drug target because any compound designed to target the HYB domain would be unlikely to react with other proteins, suggesting a naturally inherent specificity.

Statements of Invention

According to a first aspect of the invention there is provided a drug screening method comprising:

a)contacting an isolated polypeptide with a compound wherein the polypeptide comprises or consists of the following phosphotyrosine-binding domain:

TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV

H (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto wherein the following amino acids are conserved C166, C172, H185, and H190;

b)determining whether binding occurs between the polypeptide and the compound; and

c)where said binding occurs concluding said compound may be useful in preventing the degradation of proteins that bind with said polypeptide.

In the above method said sequence homology may be 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45%.

In an alternative aspect of the invention there is provided a drug screening method comprising:

a)contacting an isolated polypeptide with a compound wherein the polypeptide comprises or consists of the following phosphotyrosine-binding domain:

TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV

H (a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto;

b)determining whether binding occurs between the polypeptide and the compound; and

c)where said binding occurs concluding said compound may be useful in preventing the degradation of proteins that bind with said polypeptide.

In the above method said sequence homology may be 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

In a preferred embodiment of the invention said polypeptide comprises or consists of sequence structure:

VHFCDKCGLPlklYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE

QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV

H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23 % homologous thereto wherein the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190.

In the above method said sequence homology may be 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45%.

In a preferred embodiment of the invention said polypeptide cpmprises or consists of sequence structure:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE
QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV
H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 71% homologous thereto.

In the above method said sequence homology may be 72%, 73%, 74%, 75%, 76%,77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

More preferably still, said polypeptide comprises or consists of a phosphotyrosine-binding domain characterised by two of the following sequence structures:
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto where the following amino acids are conserved C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer.

More preferably still, said polypeptide comprises or consists of a phosphotyrosine-binding domain characterised by two of the following sequence structures:
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto, arranged as a dimer, ideally an anti-parallel dimer.

More preferably yet, said polypeptide comprises or consists of a phosphotyrosine-binding domain characterised by two of the following sequence structures:

VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE
QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV
H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23% homologous thereto where the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer.

More preferably yet, said polypeptide comprises or consists of a phosphotyrosine-binding. domain characterised by two of the following sequence structures:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE
QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV
H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least. 71% homologous thereto, arranged as a dimer, ideally an anti-parallel dimer.

Yet more preferably still, said method is undertaken using a ubiquitin 3 ligase protein or a polypeptide fragment thereof which comprises a phosphotyrosine-binding domain characterised by
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto where the following amino acids are conserved C166, C172, H185, and H190; or
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE
QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV
H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23% homologous thereto where the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190.

Yet more preferably still, said method is undertaken using a ubiquitin 3 ligase protein or a polypeptide fragment thereof which comprises a phosphotyrosine-binding domain characterised by
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH

(a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto; or

VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIE QCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV H (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 71% homologous thereto.

In yet a further preferred embodiment of the invention said protein is selected from the group comprising Hakai, ZNF645, Ligand-of-Numb protein X1 and Ligand-of-Numb protein X2.

In yet a further preferred embodiment of the invention said polypeptide or protein has the following conserved target binding residues H127 and H185. Preferably said polypeptide or protein also has the following conserved target binding residues R189 and/or Y176.

In yet a further preferred embodiment of the invention said polypeptide or protein has a 1:1 binding relationship with its target molecule. Preferably, but not exclusively, the target molecule is E-cadherin, DOK1 or cortacin.

In still yet a further preferred embodiment of the invention said polypeptide or protein has a RING domain characterized by sequence structure: VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGC SEQ ID NO:3 (106-148 a.a.).

In still yet a further preferred embodiment of the invention said polypeptide or protein comprises two zinc-finger domains, a RING domain and a minimum pTyr-binding domain that incorporates a novel, atypical zinc coordination motif. Where two polypeptides of the invention are provided the HYB domain is therefore composed of four zinc-finger domains co¬ operating to bind pTyr residues, ideally, surrounded by acidic amino acids.

In yet a further preferred embodiment of the invention under part c) where said binding occurs concluding said compound may be useful in preventing cell migration or metastasis or invasion or cancer or dysplasias or hyperplasias.

In yet a further preferred embodiment of the invention said method further includes providing reagents and conditions that enable ubiquitination to take place and determining whether ubiquitination of a protein of interest takes place in the presence of absence of said test compound and where it does not take place using this fact to demonstrate or confirm binding between said polypeptide and said compound. For example, and without limiting how this ubiquitin assay may be performed, selected cells, such as HEK 293 cells, are transfected with plasmids expressing a protein of interest and epitope-tagged ubiquitin E2 conjugating enzyme in the presence (or absence - control sample) of the said polypeptides of the invention and the test compound. Ideally also in the presence of the proteosomal inhibitor MG132. 24 hours post-transfection, cells lysates are collected using appropriate buffer. Target proteins are then precipitated using specific antibodies against those proteins. Typically the polypeptides of the invention will be included in the buffers during this assay. Precipitated proteins are analysed using the SDS-PAGE gel and immunoblotting is undertaken for detection of ubiquitination levels in the complex. The level of ubiquitination should be low in the complex where the polypeptides are included in the assay and the said polypeptides bind to the test compound thus showing the test compound is an inhibitor of same. Alternatively, GST-fusion proteins of the targets (E-Cadherine, DOK1 and cortactin) are produced and purified. GST-fusion proteins of the E3 ligases (Hakai, ZNF645, Ligand-of-Numb protein X1 and Ligand-of-Numb protein X2, or parts thereof including at least the HYB binding domain) are also produced and purified. Before the *in vitro* assay is carried out, one set of E3 ligases is incubated with said test compounds, while another set with a control solution. The ubiquitination assay is then carried out by adding the ubiquitinating buffer, E1, E2 and ATP. Upon stopping the reaction samples

are analyzed using the SDS-PAGE and western analysis. Modified proteins will be detected using the anti-Ubiquitin immunoblotting. The levels of inhibition of ubiquitination of the targets will be deduced from the control samples. The level of ubiquitination should be low in the complex where the test compound binds to the E3 ligases, or at least the HYB domain thereof, thus showing the test compound is an inhibitor of same.

In a further preferred method of the invention said binding under part c) may be determined either *in vitro, in vivo* or *in siiico* and in the latter instance having regard to the crystalline structure of the HYB domain provided in Table 1 and, ideally, the figures contained herein wherein a structure having the requisite co-ordinates and, ideally shape, is modeled for the purpose of determining binding with candidate modeled drug molecules.

According to a second aspect of the invention there is provided an isolated polypeptide selected from the group comprising:

i) TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto wherein the following amino acids are conserved C166, C172, H185, and H190;

ii) TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto;

iii) VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPV QRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTR ASLENVH (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23 % homologous thereto wherein the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190;

iv) VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPV QRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTR ASLENVH (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 71% homologous thereto;

v) two of the following sequence structures: TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV H (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto, where the following amino acids are conserved C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer;

vi) two of the following sequence structures: TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENV H (a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto arranged as a dimer, ideally an anti-parallel dimer;

vii) two of the following sequence structures: VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQ RIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRA SLENVH (a.a. 106-206) SEQ ID NO:2 or a sequence at least 23% homologous thereto where the following amino acids are conserved C109, 0 112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer;

viii) two of the following sequence structures: VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQ RIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRA SLENVH (a.a. 106-206) SEQ ID NO:2 or a sequence at least 71% homologous thereto arranged as a dimer, ideally an anti-parallel dimer;

ix) an isolated polypeptide according to i),ii), v) and vi) in combination with a RING domain characterized by sequence structure:

VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGC SEQ ID NO:3 (a.a. 106-148);

x) IHFCDKCDLPIKIYGRIIPCKHAFCYHCANLYDKVGYKVCPRCRYPVL RIEAHKRGSVFMCSIVQQCKRTYLSQKSLQAHIKRRHKRARKQVTSAS LEKVR (a.a. 54-154 ZNF645) SEQ ID N0:4 or a sequence at least 71% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190;

xi) DLVCHICLLQPLLQPLDTPCGHTFCYKCLRNFLQEKDFCPLDRKRLH FKLCKKSSILVHKLLDKLLVLCPFSSVCKDVMQRCDLEAHLKNRCPGA SHRRVALERRKTS (a.a. 47-153 LNX2) SEQ ID NO:5 or a sequence at least 25% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, €130, C133, C145 C148, C166, C172, H185, and H190; and

xii) DLICHICLQALLDPLDTPCGHTYGTLCLTNFLVEKDFCPMDRKPLVL QHCKKSSILVNKLLNKLLVTCPFREHCT-QVLQRCDLEHHFQTSCKGASHYGLTKDRKRRS (a.a. 38-144 LNX1) SEQ ID NO:6 or a sequence at least 23% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190.

According to a third aspect of the invention there is provided a crystal form of the isolated polypeptide described herein wherein said crystal is characterised by the co-ordinates and structure factors deposited at the Protein Data Bank (PDB) with the accession code 3VK6 and/or as described herein with reference to the text and figures and/or Table 3.

According to a fourth aspect of the invention there is provided a molecular target for treating a disease characterised by migration or metastasis or

invasion or a lack of cell-cell adhesion such as cancer, comprising a protein selected from the group comprising E-cadherin, DOK1 or cortactin.

As mentioned above the method of the invention may be undertaken *in silico,* this we have done using conventional software such as the software Glide, version 5.5 (Schrodinger, LLC, New York, 2009). With this *in silico* method we have demonstrated that Methotraxate Hydrate is effective at binding with the polypeptide or protein of the invention and so blocking its ability to bind E-cadherin, DOK1 or cortactin.

According to a fifth aspect of the invention there is therefore provided the use of Methotraxate Hydrate, or a derivative or salt thereof, to treat a disease characterised by migration or metastasis or invasion or a lack of cell-cell adhesion, such as cancer.

According to a sixth aspect of the invention there is therefore provided the use of Methotraxate Hydrate, or a derivative or salt thereof, in the manufacture of a medicament to treat a disease characterised by migration or metastasis or invasion or a lack of cell-cell adhesion, such as cancer.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word "comprises", or variations such as "comprises" or "comprising" is used in an inclusive sense i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

All references, including any patent or patent application, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. Further, no admission is made that any of the prior art constitutes part of the common general knowledge in the art.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking, the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including the accompanying claims and drawings). Thus, features, integers, characteristics, compounds or chemical moieties described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein, unless incompatible therewith.

Moreover, unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

The invention will now be described by way of example only with reference to the following figures:

Figure 1 shows a novel protein fold in Hakai. (A) A schematic diagram of the Hakai protein. (B) The crystal structure of Hakai (aa 106-206) reveals a dimer in an anti-parallel configuration. Each monomer contains three zinc coordination sites. Sites 1 and 2 lie in the RING domain. Site 3 is shared between the two monomers. (C) The coordination of zinc ions (purple spheres) by the RING domain of Hakai is shown for one of the monomers. (D) A schematic diagram of the cross-brace arrangement of the Hakai RING domain as shown in (C). (E) The Hakai dimer forms an intertwined configuration spanning the points indicated in circles, with the entry and exit paths shown in green and brown arrows. The zinc-interacting side chains are shown as green and brown sticks. (F) The backbone of the Hakai (aa 106-206) residues involved in intermolecular main-chain H-bonding and the zinc-coordinating side chains of adjacent monomers at the dimer interface are shown in cyan and yellow. The pink dots indicate the main-chain H-bonds; the red dots indicate the zinc coordination bonds. (G) The

monomers of the interlinked Hakai dimer are shown in surface representation and Ca trace, respectively. The Ca trace monomer enters and exits the other monomer at the red and black circles, respectively. Brown arrows show its entry and exit path. (H) A schematic diagram of the novel Hakai interlinked arrangement as shown in (G);

Figure 2 shows Hakai forms a dimer in solution. (A) A 3D ^{15}N-NOESY spectrum showing the intermolecular NOE cross-peaks of amides corresponding to residues of Hakai (aa 106-206). (B) WT Hakai (aa 106-206) and four Hakai (aa 106-206) point mutants were each separately used for gel-filtration chromatography. Their respective elution profiles were overlain and compared. (C) HA- and FLAG-tagged Hakai were overexpressed in the presence of Src in HEK293 cells. FLAG immunoprecipitates were analysed for HA-tagged Hakai. (D) A schematic representation of the point mutations made to C166, C172, H185 and H190 in Hakai. (E) Cell lysates containing WT Hakai or Hakai mutants were used to analyse the effects of Hakai dimerization on E-cadherin recognition;

Figure 3 shows Hakai domain recognizes acidic residues. (A) Y753, Y754 and Y755 (red) of E-cadherin were mutated to phenylalanine (blue) in different combinations. (B) The WT E-cadherin and the mutants shown in (A) were overexpressed in HEK293 cells with v-Src to analyse their pTyr signals. (C) HEK293 cells were co-transfected with WT E-cadherin or its mutants together with Hakai to identify the tyrosine residues recognized by Hakai. (D) The Y754-phosphorylated and non-phosphorylated E-cadherin peptides were titrated against Hakai (aa 106-206) using ITC. The top panels show the heat release profiles after baseline correction and the lower panels indicate the binding isotherms for the interactions. The dissociation constant (K_d) and binding stoichiometry (N) are shown in the table. (E) The E-cadherin aa 747-758 were each substituted with alanine. (F) The E-cadherin mutants from (E) and Hakai were co-transfected into HEK293 cells to identify the target motif on E-cadherin. (G) E-cadherin and Hakai were co-transfected into HEK293 cells. Their interaction was

analysed through immunoprecipitation of FLAG-tagged Hakai. (H) Cortactin was co-transfected into HEK293 cells with Hakai. The interaction between cortactin and Hakai was compared with that in (G). (I) Y482 and Y485 (red) were separately substituted with phenylalanine (blue). (J) WT and mutated cortactin were co-transfected into HEK293 cells with Hakai, in the absence or presence of v-Src. The pTyr signal of cortactin and its interaction with Hakai were analysed. (K) An alanine scan of cortactin aa 478-489. Each residue was substituted with alanine (blue). G484 was not mutated as glycine mutations affect the protein structure. (L) The cortactin mutants described in (K) were co-transfected into HEK293 cells with Hakai. The interaction between the cortactin mutants and Hakai was determined using immunoprecipitation;

Figure 4 shows DOK1 interacts with Hakai. (A) The sequence alignment of the different Src phosphorylation target sites in E-cadherin, cortactin and DOK1 . The acidic amino-acid residues flanking the phosphorylated tyrosine are shown in blue. (B) Hakai and DOK1 were overexpressed in HEK293 cells in the absence or presence of Src. FLAG immunoprecipitates were analysed for DOK1 interaction. (C) DOK1 was co-transfected into HEK293 cells with Hakai to study its competition with endogenous cortactin for binding to Hakai. FLAG immunoprecipitates were immunoblotted for cortactin;

Figure 5 shows target-binding amino acids of Hakai. (A) An overlay of the ^{1}H- ^{15}N-HSQC spectra of Hakai (aa 106-206) in the absence (green) or the presence (red) of an tyrosine-phpsphorylated E-cadherin peptide. (B) A graphical representation of the combined chemical shift perturbation (p.p.m.) plotted against all Hakai (aa 106-206) residues, with the cutoff at the combined chemical shift perturbation of 0.15 p.p.m. (C) The six potential E-cadherin-interacting residues in Hakai (aa 106-206) are highlighted as sticks in the ribbon representation of the crystal structure. (D) An electrostatic surface potential representation of Hakai (aa 106-206) shows that H127, Y176, H185 and R189 form part of the positively charged

pocket. (E) The interaction between E-cadherin and the Hakai mutants of the residues identified in (C) was analysed by immunoprecipitating FLAG-tagged Hakai. (F) HEK293 cells were transfected with the identified Hakai mutants, and their interaction with endogenous cortactin was studied. (G) Immunoprecipitates of either WT Hakai or the Hakai zinc-coordinating mutants were tested for interaction with endogenous cortactin. (H) A schematic representation of the Hakai dimer and the HYB domain;

Figure 6 shows the HYB domain in other proteins. (A) A comparison of the Hakai protein from amino-acid residues 127-191 and the equivalent sequence in ZNF645. (B) E-cadherin and ZNF645 were analysed for their interaction using immunoprecipitation. Hakai was used as a positive control. The dotted arrow indicates a non-specific band; the solid arrow indicates the ZNF645 band. (C) ZNF645 was overexpressed in HEK293 cells and its interaction with endogenous cortactin was analysed using immunoprecipitation. (D) Sequence alignment of LNX1 and LNX2 with Hakai and ZNF645 based on the conserved zinc-coordinating residues from Hakai aa 106-206;

Figure 7 shows the novel structure of the HYB domain. (A) Representative structures of SH2 (PDB code 1SHB), PTB (PDB code 1SHC), PKCd C2 (PDB code 1YRK) and PKM2 (PDB code 3BJF) in ligand-free forms are compared with the HYB domain. (B) The corresponding topologies of the domains in (A);

Figure 8 shows *ITC analysis Hakai (106-206) interactions with DOK1 and Cortactin* Figure 8A The Y361 phosphorylated DOK1 peptide was titrated against Hakai (aa 106 - 206) using ITC. The top panels show the heat release profiles after baseline correction and the lower panels indicate the binding isotherms for the interactions. The dissociation constant (Kd) and binding stoichiometry (N) are shown in the table. Figure 8B The cortactin peptide double phosphorylated at Y482 and Y485 was titrated against Hakai (aa 106 - 206) using ITC. The top panels show the heat release

profiles after baseline correction and the lower panels indicate the binding isotherms for the interactions. The dissociation constant (Kd) and binding stoichiometry (N) are shown in the table. Notably, these binding studies with novel cancer targets Cortacin and DOKIhave Kd values for binding of Cortacin and DOK1 to the HYB domain in the order of 28.4μM and 5.33μM, respectively, thus demonstrating the specificity of the HYB domain for these proteins and their role as targets in cancer therapy; and

Figure 9 shows the amino acid sequence structure of Hakai with the HYB domain highlighted.

METHODS

Plasmids

Mouse Hakai, human E-cadherin and avian v-Src were gifts from W Birchmeier (Max-Delbru¨ck-Center for Molecular Medicine, Germany), W Hunziker (IMCB, Singapore) and XM Cao (IMCB, Singapore), respectively. Mouse cortactin was from Addgene (Cambridge, MA) (plasmid 26722, deposited by A Weaver). Human ZNF645 and DOK1 were from Origene (Rockville, MD). Where necessary, the genes were cloned into pXJ40-HA or pXJ40-FLAG. For structural studies, Hakai constructs were cloned into pGEX6P-1 (GE Healthcare, UK). Point mutants and truncates were generated using the proofreading Pfu DNA polymerase.

Antibodies and reagents

Mouse anti-FLAG M2, rabbit anti-FLAG and anti-HA and agarose-conjugated anti-FLAG M2 beads were obtained from Sigma-Aldrich (St Louis, MO). Rabbit GST, cortactin, E-cadherin and DOK1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein-A-conjugated agarose beads were from Roche Molecular Biochemicals (Germany). Mouse anti-E-cadherin and HRP-conjugated anti-pTyr PY20

were from BD Transduction Laboratories (Lexington, KY). Mouse anti-b-actin was obtained from Abeam (Cambridge, MA).

Cell lines and transfection

HEK293 cells were purchased from ATCC (Manassas, VA) and maintained as described (Yusoff et al, 2002). Transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting were carried out as described (Yusoff et al, 2002) with the following modifications. HEK293 cells were harvested 24 h post-transfection with a lysis buffer containing protease inhibitors (Roche) and 1 mM Na_3V0_4. Immunoprecipitations were performed using agarose-conjugated anti-FLAG or protein-specific antibodies followed by incubation with protein-A-conjugated agarose beads at 41C.

Liquid chromatography-mass spectrometry/mass spectrometry

Immunoprecipitates were separated by SDS-PAGE, and stained with Coomassie Blue G250. Protein bands were excised and washed with 25 mM ammonium bicarbonate (ABB) in 50% acetonitrile (ACN) buffer thrice. The proteins in the gel were reduced with 10 mM DTT in 25 mM ABB buffer, alkylated with 5 mM iodoacetamide, dehydrated and digested with trypsin overnight. After in-gel digestion, the solution was transferred to a clean tube and sonicated for 30 min in the presence of 50 ml 50% ACN and 5% acetic acid for protein extraction. This extraction procedure was repeated three times; the pooled extracts were dried with a vacuum concentrator. The samples were processed and analysed as described (Zhang et al, 2010) using a LTQ-FT ultra mass spectrometer.

For each experiment, MS/MS (dta) spectra were extracted from the raw data files using the extract_msn program in Biowork 3.3 (ThermoFinnigan). The extracted dta files were combined into a single file in the Mascot generic file (mgf) format. Except for the conversion of precursor mass from MH þ in dta to m/z in mgf, the fragment ion m/z and intensity values were used as determined. Proteins were identified by searching the combined data against the IPI human database (downloaded on 25 November 2009, including 86 845 sequences and 35122 444 residues) via an in-house Mascot server (version 2.2.07). Two missing cleavages were allowed. Precursor ion and MS/MS fragment ion error tolerances were set to o10 p.p.m. and o0.8 Da, respectively. A protein was accepted as a true positive if it had a significant score (Po0.05) and at least two unique peptides.

Protein purification and gel-filtration chromatography

The GST-tagged Hakai (aa 106-206) constructs were expressed in Escherichia coli BL21 (DE3) and purified using glutathione-conjugated sepharose (GE Healthcare). The GST-tag was cleaved using GST-PreScission Protease (GE Healthcare) and the proteins were applied to a Superdex 75 size-exclusion column (GE Healthcare) equilibrated using 10 mM Bis-Tris, pH 6.5, 250 mM NaCl and 5 mM DTT and pre-calibrated using a gel-filtration standard (Bio-Rad).

NMR spectroscopy and chemical shift perturbation analysis

$^{15}N/^{13}C$-labelled Hakai (aa 106-206) was obtained from cultures grown in M9 media supplemented with ^{15}N-labelled ammonium chloride and $^{13}e\neg$ labelled glucose as the sole nitrogen and carbon sources, respectively. The labelled proteins were purified as described above. NMR spectra were acquired at 298 K in an 800-MHz NMR spectrometer (Bruker, Karlsruhe, DE). The backbone assignment was obtained using standard ^{15}N-edited HSQC, HNCACB and CBCA (CO)NH experiments; side chains were

assigned using standard 3D-T0CSY, 3D-N0ESY and HCCH-TOCSY experiments. NMR data were processed using NMRPipe (Delaglio et al, 1995) and analysed by NMRView (Johnson and Blevins, 1994).

For the chemical shift perturbation analysis, the 2D ^{1}H-^{15}N-HSQC spectra for the ^{15}N-labelled Hakai (aa 106-206) were acquired in the absence or presence of the phosphorylated E-cadherin peptide. Perturbed residues on Hakai were assigned by super-imposing the two HSQC spectra.

Crystallization and structure determination

SelMet-substituted Hakai (aa 106-206) was expressed in a methionine auxotroph (Doublie, 1997) and purified as described above. SelMet incorporation was verified by MALDI-TOF.

SelMet Hakai (aa 106-206) crystals were grown at 289 K by the hanging drop vapour diffusion method. The protein (30 mg/ml) was mixed with an equal volume of reservoir solution (140 mM Li_2S0_4; 100 mM Tris, pH 7.8; 15 mM $Na_2S_20_3$; 20-22% PEG 5000 MME; 1% isopropanol and 20-25% ethylene glycol). A complete SAD data set was collected to 1.9 A resolution at the synchrotron beamlines (NSLS, Brookhaven National Laboratory and the National Synchro-tron Radiation Research Center [NSRRC], Taiwan) using a Quantum4-CCD detector (Area Detector Systems Corp., Poway, CA). The datasets were processed and scaled using HKL2000 (Otwinowski and Minor, 1997). The crystals belonged to space group $P6_222$ with a = 64.66 A, b = 64.66 A, c = 121.04 A, and contained one molecule in the asymmetric unit.

All four expected selenium sites in the asymmetric unit were located by SOLVE (Terwilliger and Berendzen, 1999). Initial phases were developed by RESOLVE (Terwilliger, 2003); the overall figure of merit was improved to 0.83, and over 90% of the molecule was built automatically. The remaining parts of the model were built manually using COOT (Emsley and Cowtan,

2004) and alternatively refined by CNS (Brunger et al, 1998) and PHENIX (Adams et al, 2002). The final model was refined to a 1.9-A resolution with an R-factor of 0.2175 (Rf_{ree} = 0.2396) and analysed using PROCHECK (Laskowski et al, 1993). AILstructure-related figures were prepared using PyMOL (DeLano, 2002).

Circular dichroism spectrometry

Far UV spectra (260-190 nm) of Hakai (aa 148-206) and Hakai (aa 106-206) and its mutants were measured using a Jasco J-810 spectropolarimeter in phosphate buffer (pH 7.5) at room tempera-ture using a 0.1-cm path length and stoppered cuvettes. Six scans were recorded, averaged and the baseline subtracted.

Dynamic light scattering

Dynamic light scattering studies were carried out on a DynaPro Light Scattering instrument (Protein Solutions, USA) at a protein concentration of 2 mg/ml, in a buffer containing 10 mM Bis-Tris pH 6.5, 250 mM NaCI and 5 mM DTT.

Isothermal titration calorimetry

Phosphorylated and non-phosphorylated peptides of E-cadherin (residues 749-761); phosphorylated peptides of DOK1 (residues 356-366) and Cortactin (residues 477-489) were titrated at a molar concentration of 800 mM against 100 mM of Hakai (aa 106- 206) dimer in a VP-ITC microcalorimeter (Microcal, Northhampton, UK) at 293 K. The titrations were carried out using 30 10-ml injections of the appropriate peptide into the sample cell containing Hakai (aa 106-206) and the data were analysed with a one-site binding model using the Origin software package v7.0 supplied by Microcal. All measurements were repeated twice.

Accession number

The coordinates and structure factors have been deposited at the Protein Data Bank (PDB) with the accession code 3VK6.

RESULTS

A novel protein fold in Hakai

We experimentally established that the minimum E-cadherin phosphotyrosine-binding sequence in Hakai was contained within amino acids 148-206 (aa 148-206) (Supplementary Figure S1A-G). Circular dichroism analysis however revealed that purified Hakai (aa 148-206) was unstructured (Supplementary Figure S1H), and was not suitable for crystallization. Therefore, the Hakai sequence spanning amino-acid residues 106-206 (aa 106-206) that contained both the RING domain and the minimum pTyr-binding domain, as represented schematically in Figure 1A, was purified and crystallized.

The crystal structure of Hakai (aa 106-206) was solved at 1.9 Å resolution (Figure 1B). The striking feature of the crystal structure was the formation of a dimer from paired, anti-parallel Hakai (aa 106-206) monomers. Each monomer consisted of an N-terminal RING domain, followed by the C-terminal atypical zinc-binding domain that is contained within the experimentally derived minimum pTyr-binding domain. Furthermore, each monomer contained three zinc ions at three distinct sites. One zinc ion coordinated with the atypical zinc-binding domains of both monomers (Figure 1B). The Hakai RING domain (residues 106-148) adopted a typical RING domain fold stabilized by co-ordinating with two zinc ions, forming a cross-brace arrangement (Figure 1C and D). The zinc-coordinating residues in the RING domain are also indicated in Figure 1D.

The uniqueness of the Hakai (aa 106-206) region was revealed when the

structure was compared with other proteins in the PDB (Protein Data Bank) using the DALI server (http://ekhidna.biocenter.helsinki.fi/dali_server/). The results show that only the RING domain of Hakai is structurally similar to RING domains of other proteins. There is, however, no similarity beyond amino-acid residue 159 of the Hakai minimum pTyr-binding domain, which is located on the dimerization interface. The minimal pTyr-binding domain of Hakai adopts a novel, three-dimensional fold and contains three b-strands (b4, b5 and b6) and a C-terminal a-helix. The b-strands b4, b5 and b6 were in an extended configuration and formed anti-parallel b-sheets with the corresponding b-strands of the monomeric partner during homodimerization (Figure 1E). The atypical zinc-finger motif within this region is formed by two histidine residues (H185 and H190) and one cysteine residue (C172) from one monomer and a second cysteine residue (C166) from the adjacent monomer (Figure 1F; Supplementary Figure S2), unlike a classical C2H2 zinc finger (ZnF). Although the Hakai (aa 106-206) region in each monomer fulfils the required criteria of the zinc coordination consensus pattern of cysteine and histidine residues [C-x(5)-C-x(12)-H-x(4)-H], it is not capable of forming the zinc coordination sphere by itself, since a second cysteine residue is required of its anti-parallel monomeric partner (Figure 1F). Therefore, each dimer contains two atypical zinc-finger motifs.

The crystal structure of Hakai also shows that the two Hakai monomers intertwine through a stretch of residues ranging from F164 to Y176 during dimerization, resulting in the formation of three β-sheets based on 12 main-chain hydrogen bonds (Figure 1E-H). This novel interlinked con-figuration and the two atypical zinc ion interactions at the dimer interface are unique features of this distinctive homo-dimeric assembly. A surface area of approximately 1650A^2 of each monomer (or -21% of each monomer surface) was formed at the dimer interface of Hakai (aa 106-206), with 34 hydrogen bond contacts between the monomers, as analyzed by the PISA server (Krissinel and Henrick, 2007).

Hakai forms a dimer in solution

A previous study alluded to the similarity between the Hakai (aa 106-206) polypeptide with the dimerization domain of the V(D)J recombination-activating protein RAG1 (Fujita et al, 2002). In addition to the crystal structure, the inter-molecular NOE cross-peaks corresponding to the amides of residues at the dimer interface also indicates that Hakai (aa 106-206) forms a dimer in solution (Figure 2A). Significantly, the structures of the dimerization interface of the RAG1 domain (Bellon et al, 1997) and Hakai (aa 106-206), as described in this study, are completely dissimilar. The formation of Hakai (aa 106-206) dimers in solution is also supported by the results obtained through dynamic light scattering, which show an apparent molecular weight of 24.1 kDa, twice than that of the monomer.

We next examined whether zinc coordination is necessary for the dimerization of Hakai and its ability to interact with its target. We first investigated whether mutations of the zinc-coordinating residues within the minimum pTyr-binding do-main (C166, C172, H185 and H190) would affect the proposed Hakai dimerization. The Hakai (a.a. 106-206) polypeptides containing point mutations at these residues were separated on a calibrated gel-filtration column. Their gel-filtration elution profiles show that each point mutant had an apparent molecular weight equivalent to a monomeric unit of wild-type (WT) Hakai (a.a. 106-206) protein (B12 kDa), whereas WT Hakai eluted as a dimer (Figure 2B). Furthermore, circular dichroism performed using all the Hakai (a.a. 106-206) mutants ascertained that each one has maintained a well-defined secondary structure. These findings suggest that each zinc-coordinating residue is instrumental in forming the dimer interface.

Having determined that Hakai (a.a. 106-206) dimerizes in solution, we next examined if this occurs with full-length proteins. Full-length FLAG-tagged Hakai was observed to bind to its HA-tagged counterpart (Figure 2C), indicating that dimerization also occurs between the full-length proteins. To

further determine whether Hakai dimerization is required for its function in binding its targets, the full-length proteins containing alanine point mutations of the zinc-coordinating residues (Figure 2D) were tested for their ability to bind to tyrosine-phosphorylated E-cadherin. HEK293 cells were used for such studies as they did not express detectable levels of endogenous E-cadherin, which could have interfered with the mammalian cellular assays used. The evidence presented in Figure 2E indicates that none of the four Hakai point mutants interacted with tyrosine-phosphorylated E-cadherin. The collective results therefore show that zinc coordination is necessary for both dimerization of Hakai and its subsequent function in interacting with its target.

Hakai domain recognizes acidic residues

Having established the novel characteristics of the Hakai zinc-coordinated homodimer, we sought to identify the target motif of this new domain. At this point, the only described target motif was in Src-phosphorylated E-cadherin. Within this motif, two (Y755 and Y756 in mouse; Y753 and Y754 in humans) of three consecutive tyrosine residues were reported to be involved in the interaction with Hakai (Fujita et al, 2002). To analyse the relative contributions of the three tyrosine residues in binding to Hakai, mutations were made to the tyrosine residues (Figure 3A). To determine which of the tyrosines were phosphorylated, we analysed the patterns of the tyrosine phosphorylation of the point mutants after v-Src activation. The pTyr signals shown in Figure 3B indicate that all three adjacent tyrosines were phosphorylated.

We next examined the importance of the three tyrosine residues in E-cadherin for its interaction with Hakai. The E-cadherin mutants used in the earlier experiment were analysed for, their potential to bind to WT Hakai. The results shown in Figure 3C indicate that Y754 is the only tyrosine significantly involved in binding; each mutant containing a substitution in this position did not bind Hakai, whereas all other mutants showed

significant binding. These combined results also show that while Src binds to and phosphorylates most of the E-cadherin mutants, all the mutants with a Y754F substitution do not bind to Hakai, even when phosphorylated. This implies that the interaction between Hakai and E-cadherin depends on the direct recognition of specific pTyr residues on E-cadherin by Hakai. To verify the necessity of Y754 phosphorylation for the interaction between E-cadherin and Hakai, isothermal titration calorimetry (ITC) was performed using phosphorylated and non-phosphorylated E-cadherin peptides corresponding to a.a. 749-761 with Hakai (aa 106-206). The results in Figure 3D show that binding occurred only with the phosphorylated peptide. Furthermore, the results also indicate that only one E-cadherin peptide binds to the Hakai (aa 106-206) dimer at any one time.

In a similar manner, we analysed the importance of the amino-acid residues flanking the tyrosines for the interaction between the two proteins. via an alanine scan (Figure 3E). The immunoprecipitation results in Figure 3F show that there were profound contributions from the aspartic acid D756 and glutamic acid E757, and significant contributions from valine V752 and aspartic acid D750. Consequently, a cluster of negative charges from the acidic amino acids is formed around the centrally binding tyrosine 754 of E-cadherin.

To verify the motif recognized by Hakai, additional target proteins were identified. This identification was accomplished by analysing Hakai-binding proteins phosphorylated by Src using mass spectrometry. A list of the proteins obtained from a typical experiment is appended in Supplementary Figure S4A and B, in which cortactin was identified as a potential target. Mouse cortactin is phosphorylated by Src primarily on Y482 and Y485. Interestingly, these two tyrosines are also surrounded by several acidic residues. We investigated whether Hakai binds to Src-phosphorylated cortactin, as well as the importance of Y482, Y485 and their flanking residues in this interaction. In addition to the WT proteins, phenylalanine substitution and alanine scan experiments were also per-formed on

cortactin as described for E-cadherin. The results for cortactin mirror those obtained for E-cadherin. Like E-cadherin, cortactin interacts with Hakai only when phosphorylated by Src (Figure 3G and H). Phenylalanine substitutions of the Src-phosphorylated tyrosine residues revealed that Y482 is the main tyrosine residue involved in Hakai binding (Figure 3 1 and J). Furthermore, the acidic residues (E478, D480 and E486) surrounding Y482 contributed profoundly to the interaction between Hakai and E-cadherin, and significant contribution was also observed from S487 (Figure 3K and L).

To further verify that Hakai targets pTyrs of Src substrates with surrounding acidic residues, another Src substrate was selected: DOK1 , which also contains pTyrs with adjacent acidic groups. One such particular tyrosine residue was found to be a primary phosphorylation site of Src (Figure 4A). The results show that DOK1 interacts with Hakai. Furthermore, DOK1 competed with endogenous cortactin for Hakai, implying that DOK1 and cortactin bind Hakai on the same site (Figure 4A-C).

Target-binding amino acids of Hakai

To identify the residues in Hakai within a.a. 106-206 necessary for its interaction with E-cadherin, 2D ^{1}H- ^{15}N-HSQC spectra of ^{15}N-labelled Hakai (aa 106-206) were acquired in the absence and presence of a pTyr peptide derived from amino-acid residues 749-761 of E-cadherin. Based on the changes in the NMR spectrum of Hakai (aa 106-206), several residues underwent perturbations in chemical shift (Figure 5A and B). A minimum criterion of a chemical shift difference $Ddp._{p'm'}$.40. 15 p.p.m. was applied. Five residues were identi-fied (Y176, H185, N187, H 188 and R 189), which resided in the Hakai minimum pTyr-binding domain, whereas a sixth residue (H127) was from the RING domain (Figure 5C). However, the crystal structure shows that only four of these six residues reside in a close three-dimensional spatial proximity. While H127, Y176, H185 and R189 face the interior of the E-cadherin-binding site (Figure 5C) and form a

positively charged pocket, N187 and H188 face outward and are not part of the pocket (Figure 5D).

These residues identified through *in vitro* peptide-domain binding assays were then further tested by expressing full-length point mutants in HEK293 cells. The immunoprecipitation results shown in Figure 5E indicate that the residues identified in the NMR analysis also abrogated binding when mutated, with the exception of residues N187 and H188. As expected, the required residues were situated on the interior of the target-binding domain, whereas the non-binding residues, N187 and H188, were on the exterior. Similar results were obtained with experiments using cortactin (Figure 5F), demonstrating the importance of these Hakai residues. Furthermore, dimerization of Hakai is also required, as with E-cadherin, as cortactin was unable to bind to Hakai containing mutations to its zinc-coordinating residues (Figure 5G).

Based on the evidence obtained, it can be concluded that two Hakai monomers interact in an anti-parallel manner to form a dimer via the interlinked zinc-coordinating domain. This domain binds pTyrs flanked by acidic amino acids in Src substrates. The target-binding domain resulting from this dimerization process represents the functional Hakai phosphotyrosine-binding domain, henceforth referred to as the HYB (Hakai pY-binding) domain (Figure 5H).

The HYB domain in other proteins

We next investigated whether the HYB domain is found in other proteins. Literature and database searches revealed that the testis-specific ubiquitin E3 ligase ZNF645 exhibited high-sequence homology (71%) with Hakai, as shown in Figure 6D. The sequence homology between Hakai and LNX2 and 1 were 25% and 23%, respectively. When the homology was investigated for only the novel binding sequence (a.a. 159-206) the testis-specific ubiquitin E3 ligase ZNF645 exhibited high-sequence homology (76%) with Hakai, as shown in Figure 6D. The sequence homology

between the binding sequence Hakai (a.a. 159-206) and LNX2 and 1 were 31% and 37%, respectively. We therefore questioned whether ZNF645 could also interact with E-cadherin and cortactin. The results in Figure 6B show that ZNF645 bound to v-Src-phosphorylated E-cadherin but not to cortactin (Figure 6C). This result implies that although there is significant homology between Hakai and ZNF645, they are likely to have their own sets of targets due to the differences in their sequences between the key zinc-coordinating residues.

Based on the key amino-acid residues involved in zinc coordination and binding in HYB, we searched the NCBI database to analyse gene origins and protein homologies (Table 2). Two interesting results emerged. First, a comparison of the species distribution of the Hakai and ZNF645 gene products indicated that the latter, found only in primates, is most likely a recent copy of the former. Second, ZNF645 is an intronless gene, implying that it is a retrotransposed copy of Hakai.

Further database searches based on the conserved zinc-coordinating cysteine and histidine residues within the HYB domain showed that a similar series of residues is present in LNX1 and LNX2 (Figure 6D). This implies that the HYB domain may be distributed in other proteins, although the latter observation requires experimental confirmation.

Novel structure of the HYB domain

The structures of the five pTyr-binding domains that have been discovered to date are illustrated in Figure 7A and B. All of the domains, except for the HYB domain, are contained within one monomer. The HYB domain consists of a pair of monomers arranged in an anti-parallel configuration and is composed of two RING and two atypical zinc-coordinating domains. From this comparison, it is apparent that all five of these pTyr domains have completely different structures, with different strategies to recognize tyrosine phosphorylation.

References

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Bellon SF, Rodgers KK, Schatz DG, Coleman JE, Steitz TA (1997) Crystal structure of the RAG1 dimerization domain reveals multi-ple zinc-binding motifs including a novel zinc binuclear cluster.Nat Struct Biol 4: 586-59.

Table 1 Crystallographic data and refinement statistics

	SeMet-SA
Data collection	
Space group	$P6_222$
Cell dimensions	
a, *b*, *c* (Å)	64.66, 64.66, 121.04
( )	90.00, 90.00, 120.00
Wavelength (Å)	0.979
Resolution (Å)	50-1.9 (1.97-1.90)
Observed reflections	509428
Unique reflections	22371
R_{sym} [a]	0.052 (0.428)
/ / /	21.3 (12.6)
Completeness (%)	99.7 (100)
Redundancy	22.8 (22.6)
Refinement	
Resolution (Å)	25.2-1.9
Reflections (working set / test set)	19327/2153
R_{work} [b] / R_{free} [c]	0.2175/0.2396
No. atoms	
Protein	756
Zn^{2+}	3
Water	73
B-factors (Å^2)	
Protein	38.75
Zn^{2+}	30.46
Water	44.06
Ramachandran statistics	
Most favorable regions (%)	90.2
Additional allowed regions (%)	9.8
Generously allowed regions (%)	0.00
Disallowed regions (%)	0.00
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles ( )	1.202

Values in parentheses are for highest-resolution shell.

[a] $R_{merge} = \sum|I_i - \langle I \rangle| / \sum|I_i|$, where I_i is the intensity of the i-th measurement, and $\langle I \rangle$ is the mean intensity for that reflection.

[b] $R_{work} = \sum||F_{obs}| - |F_{calc}|| / \sum|F_{obs}|$, where F_{calc} and F_{obs} are the calculated and observed structure factor amplitudes, respectively.

[c] R_{free} = as for R_{work}, but was calculated using 10% of data excluded from refinement.

Table 2

List of proteins with sequences that potentially match the region of Hakai from amino acid residues 127 to 189.

	Species	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
HAKAI	Homo sapiens (Human)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Bos taurus (Cattle)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Macaca mulatta (Rhesus monkey)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGS.LFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Rattus norvegicus (Norway rat)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Ailuropoda melanoleuca (Giant panda)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Mus musculus (House mouse)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Equus caballus (Horse)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Canis lupus familiaris (Dog)	HVFCYDCAILHEKKG DKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Pan troglodytes (Chimpanzee)	HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Gallus gallus (Chicken)	HVFCYDCAILHEKKGDKMCPGCNEPVQRIEQCVRGSLFMCSIVQGCKRT YLSQRDLQ AHINHRH
	Xenopus laevis (African clawed frog)	HVFCYDCALMHEKKADKLCPGTLVEESTDTFKRMSCNDPVQRIEQCARG SLFMCSIV QGCKRTYLSQRDLQAHINHRH
	Danio rerio (Zebrafish)	HVFCYDCAVYYE KKCDKMCPGLSLYSCTDPVQRIEQCQRGSLFMCNIVQ GCKRTYLS QRDLQAHINHRH
	Salmo salar (Atlantic salmon)	HVFCYDCALLHEKKMEKMCPGLTLYSCTDPVQRIEQCLRGLLYMCSIVP GCKRTYLS QRDLQAHVNHRH
	Harpegnathos saltator (Jerdon's jumping ant)	HVFCLSCA ---- KREDKVCPRCMEKVSRVEQTGLGTVFMCTHGGTRYGNTGCRRTYLSQRD LQAHINHR H
	Camponotus floridanus (Carpenter ant)	HVFCLSCA — -- KREDKVCPRCMEKVSRVEQTGLGTVFMCTHGGTRYGNAGCRRTYLSQRD LQAHINHR H
	Tetraodon nigroviridis (Green pufferfish)	HVFCYDCALLHEKKGEKMCPGLTLYNCTDPVQRIEQCQRGSLYMCSVVP GCKRTYLS QRDLQAHVNHRH
	Anopheles darlingi (Mosquito)	HVFCLRCA --- RSETLKMCPRCKEKVVRVEQTALGTVFMCTHGGTRYGNTGCRRTYLSQR DLQAHINH RH
	Drosophila grimshawi (Fruit fly)	HVFCLKCA --- RAEPIKCCPRCNDKVLRVEQSGLGTVFMCTHGGSRYGSTGCRRTYLSQR DLQAHINH RH
	Tribolium castaneum (Red flour beetle)	HVFCLSCG ---- KQEQKQCPRCLEKVSRVEQTGLGTVFMCTHGGTRYGSSGCRRTYLSHRD LQAHINHR H
	Papilio xuthus (Asian swallowtail butterfly)	HVFCLSCA ---- RSDHTHCPRCREKVLRVEQTGLGTVFMCTHSGTRYGNTGCRRTYLSQRD LQAHINHR H
	Hydra magnipapillata (Freshwater hydrozoan)	HVFCLSCA ---- ENSNGECVRCEERIDRIEPATIGQIFVCSFGGNRNITSGCRRSYLSQRD LIAHIRHR H

	Species	HAFCYHCANLYDKVGYKVCPRCRYPVLRIEAHKRGSVFMCSIVQQCKRT YLSQKSLQ AHIKRRH
ZNF645	Homo sapiens (Human)	HAFCYHCANLYDKVGYKVCPRCRYPVLRIEAHKRGSVFMCSIVQQCKRT YLSQKSLQ AHIKRRH
	Macaca fascicularis (Cynomolgus Monkey)	HAFCYNCANLYDKIGYKICPRCSYPVLRIEEHKRGSVFMCSVVQGCKRT YLSQKSLQ AHIKRRH
	Macaca mulatta (Rhesus monkey)	HAFCYNCANLYDKIGYKICPRCSYPVLRIEEHKRGSVFMCSVVQGCKRT .YLSQKSLQ AHIKRRH
	Pongo abelii (Sumatran orangutan)	HAFCYDCANLDDKIGYKICPRCRYPVLRIEEHKRGSVFMCSVVPQCKRT YLSQKSLQ AHIKRRH

Table 3

```
HEADER    LIGASE                                  09-NOV-ll   3VK6
TITLE     CRYSTAL STRUCTURE OF A PHOSPHOTYROSINE BINDING DOMA IN
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE : E3 UBIQUITIN-PROTEIN LIGASE HAKAI ;
COMPND   3 CHAIN: A;
COMPND   4 FRAGMENT : PHOSPHOTYROSINE BINDING DOMAIN, UNP RESIDUES 106-
206;
COMPND   5 SYNONYM: CASITAS B-LINEAGE LYMPHOMA-TRANSFORMING SEQUENCE-
LIKE
COMPND   6 PROTEIN 1,.E-CADHERIN BINDING PROTEIN E7, C-CBL-LIKE
PROTEIN 1;
COMPND   7 EC : 6.3.2.-;
COMPND   8 ENGINEERED: YES
SOURCE    MOL_ID: 1;
SOURCE   2 ORGANISM_SCIENTIFIC: MUS .MUSCULUS;
SOURCE   3 ORGANISM_COMMON: MOUSE;
SOURCE   4 ORGANISM_TAXID: 100 90;
SOURCE   5 GENE: CBLLl, HAKAI;
SOURCE   6 EXPRESSION_SYSTEM: ESCHERICHIA COLI;
SOURCE   7 EXPRESSION_SYSTEM_TAXID: 562;
SOURCE   8 EXPRESSION_SYSTEM__STRAIN: BL21 (DE3);
SOURCE   9 EXPRESSION_SYSTEM_VECTOR_TYPE: PLASMID;
SOURCE  10 EXPRESSION_SYSTEM_PLASMID: PGEX-6P-1
KEYWDS    HYB, PHOSPHOTYROSINE BINDING DOMAIN, LIGASE
EXPDTA    X-RAY DIFFRACTION
AUTHOR    J.SIVARAMAN, M.MUKHERJEE
REVDAT   1   25-JAN-12 3VK6    0
JRNL        AUTH
M.MUKHERJEE, S .Y.CHOW, P .YUSOFF, J.SEETHARAMAN, C.NG, S .SINNIAH,
JRNL        AUTH 2
X.W.KOH,N.F.M.ASGAR, D.LI, D .YIM, R.A.JACKSON, J .YEW, J.QIAN,
JRNL        AUTH 3 A.IYU, Y .P .LIM, X.ZHOU, S.K.SZE, G.R.GUY, J.SIVARAMAN
JRNL        TITL   STRUCTURE OF A NOVEL PHOSPHOTYROSINE-BINDING
DOMAIN IN HAKAI
JRNL        TITL 2 THAT TARGETS E-CADHERIN
JRNL        REF    EMBO J .                                    2012
JRNL        REFN                    ESSN 1460-2075
JRNL        DOI    10. I038/EMBOJ.2011.4 96
REMARK   2
REMARK   2 RESOLUTION.    1.90 ANGSTROMS.
REMARK   3
REMARK   3 REFINEMENT.
```

```
REMARK   3   PROGRAM      : PHENIX  (PHENIX. REFINE:  1.6_289)
REMARK   3   AUTHORS      : PAUL ADAMS, PAVEL AFONINE ,VICENT CHEN, IAN
REMARK   3                : DAVIS, KRESHNA GOPAL, RALF GROSSE-
REMARK   3                : KUNSTLEVE, LI-WE I HUNG, ROBERT IMMORMINO,
REMARK   3                : TOM IOERGER, AIRLIE MCCOY, ERIK MCKEE, NIGEL
REMARK   3                : MORI ARTY, REETAL PAI ,RANDY READ, JANE
REMARK   3                : RICHARDSON, DAVID RICHARDSON, TOD ROMO, JIM
REMARK   3                : SACCHETTINI, NICHOLAS SAUTER, JACOB SMITH,
REMARK   3                : LAURENT STORONI,TOM TERWILLIGER, PETER
REMARK   3                : ZWART
REMARK   3
REMARK   3    REFINEMENT  TARGET   ML
REMARK   3
REMARK   3  DATA USED IN REFINEMENT.
REMARK   3   RESOLUTION RANGE HIGH (ANGSTROMS)  : 1.90
REMARK   3   RESOLUTION RANGE LOW  (ANGSTROMS)  : 25.23
REMARK   3   MIN (FOBS/SIGMA_FOBS)              : 0.240
REMARK   3   COMPLETENESS FOR RANGE        (%)  : 95.8
REMARK   3   NUMBER OF REFLECTIONS              : 21478
REMARK   3
REMARK   3  FIT TO DATA USED IN REFINEMENT.
REMARK   3   R VALUE      (WORKING + TEST SET)- : 0.222
REMARK   3   R VALUE             (WORKING SET)  : 0.219
REMARK   3   FREE R VALUE                       : 0.242
REMARK   3   FREE R VALUE TEST SET SIZE . (%)   : 10.020
REMARK   3   FREE R VALUE TEST SET COUNT        : 2153
REMARK   3
REMARK   3  FIT TO DATA USED IN .REFINEMENT  (IN BINS) .
REMARK   3   BIN   RESOLUTION  RANGE   COMPL .    NWORK  NFREE    RWORK
RFREE
REMARK   3     1 25.2305  -  4.0821     0.97      1970    217   0.2219
0.2351
REMARK   3     2  4.0821  -  3.2422     0.99      1999    225   0.1990
0.2212
REMARK   3     3  3.2422  -  2.8330     0.98      1991    226   0.2246
0.2724
REMARK   3     4  2.8330  -  2.5743     0.97      1954    213   0.2294
0.2498
REMARK   3     5  2.5743  -  2.3899     0.96      1934    212   0.217-3
0.2349
REMARK   3     6  2.3899  -  2.2491     0.96      1947    213   0.2058
0.2143
REMARK   3     7  2.2491  -  2.1365     0.96      1932    220   0.2156
0.2559
REMARK   3     8  2.1365  -  2.0436     0.95      1921    216   0.2249
0.2400
REMARK   3     9  2.0436  -  1.9649     0.93      1874    206   0.2372
0.2645
REMARK   3    10  1.9649  -  1.8971     0.89      1803    205   0.2445
0.2554
REMARK   3
REMARK   3  BULK SOLVENT MODELLING .
REMARK   3   METHOD USED          : FLAT BULK SOLVENT MODEL
REMARK   3   SOLVENT RADIUS       : 1.11
REMARK   3   SHRINKAGE RADIUS     : 0.90
REMARK   3   K_SOL                : 0.42
REMARK   3   B_SOL                : 60.07
REMARK   3
```

```
REMARK   3  ERROR  ESTIMATES.
REMARK   3   COORDINATE  ERROR  (MAXIMUM-LIKELIHOOD   BASED)      : 0.230
REMARK   3   PHASE  ERROR  (DEGREES,  MAXIMUM-LIKELIHOOD   BASED)  : 22. 600
REMARK   3
REMARK   3  B VALUES.
REMARK   3   FROM  WILSON  PLOT            (A**2)  : 31.95
REMARK   3   MEAN  B VALUE       (OVERALL,  A**2)  : 38 .94
REMARK   3   OVERALL  ANISOTROPIC   B VALUE.
REMARK   3    Bll  (A**2)  : 3 .48460
REMARK   3    B22  (A**2)  : 3 .48460
REMARK   3    B33  (A**2). : -6 .96930
REMARK   3    B12  (A**2)  : 0 .00000
REMARK   3    B13  (A**2)  : -0.00000
REMARK   3    B23  (A**2)  : 0 .00000
REMARK   3
REMARK   3  TWINNING  INFORMATION.
REMARK   3   FRACTION : NULL
REMARK   3   OPERATOR:  NULL
REMARK   3
REMARK   3  DEVIATIONS  FROM  IDEAL  VALUES .
REMARK   3                   RMSD           COUNT
REMARK   3   BOND       :  0.008            771
REMARK   3   ANGLE      :  1.172           1031
REMARK   3   CHIRALITY  :  0.081            108
REMARK   3   PLANARITY  :  0.006            133
REMARK   3   DIHEDRAL   : 13.796            297
REMARK   3
REMARK   3  TLS  DETAILS
REMARK   3   NUMBER  OF TLS  GROUPS   : NULL
REMARK   3
REMARK   3  NCS  DETAILS
REMARK   3   NUMBER  OF NCS  GROUPS  : NULL
REMARK   3
REMARK   3  OTHER  REFINEMENT  REMARKS:  SF FILE  CONTAINS  FRIEDEL  PAIRS.
REMARK   4
REMARK   4 3VK6  COMPLIES  WITH  FORMAT  V . 3.30,  13-JUL-ll
REMARK  100
REMARK  100 THIS  ENTRY  HAS  BEEN  PROCESSED  BY PDBJ  ON 22-NOV-ll.
REMARK  100 THE  RCSB  ID CODE  IS  RCSB095146.
REMARK  200
REMARK  200 EXPERIMENTAL   DETAILS
REMARK  200  EXPERIMENT  TYPE                  : X-RAY  DIFFRACTION
REMARK  200  DATE  OF DATA  COLLECTION         : 20-JUL-10
REMARK  200  TEMPERATURE             (KELVIN)  : 100
REMARK  200  PH                                : 8
REMARK  200  NUMBER  OF CRYSTALS  USED         : 1
REMARK  200
REMARK  200  SYNCHROTRON                (Y/N)  : Y
REMARK  200  RADIATION  SOURCE                 : NSLS
REMARK  200  BEAMLINE                          : X4A
REMARK  200  X-RAY  GENERATOR  MODEL           : NULL
REMARK  200  MONOCHROMATIC   OR LAUE     (M/L) : M
REMARK  200  WAVELENGTH  OR RANGE          (A) : 0.979 '
REMARK  200  MONOCHROMATOR                     : SAGITALLY  FOCUSED  SI (111)
REMARK  200  OPTICS                            : NULL
REMARK  200
REMARK  200  DETECTOR  TYPE                    : CCD
REMARK  200.  DETECTOR  MANUFACTURER           : ADSC  QUANTUM  4
```

```
REMARK 200  INTENSITY-INTEGRATION  SOFTWARE:  HKL-2000
REMARK 200  DATA SCALING SOFTWARE          : HKL-2000
REMARK 200
REMARK 200  NUMBER OF UNIQUE REFLECTIONS   : 21480
REMARK 200  RESOLUTION RANGE HIGH      (A) : 1.900
REMARK 200  RESOLUTION RANGE LOW       (A) : 50.000
REMARK 200  REJECTION CRITERIA   (SIGMA(I)) : 2.000
REMARK 200
REMARK 200 OVERALL .
REMARK 200  COMPLETENESS FOR RANGE     (%) : 99.7
REMARK 200  DATA REDUNDANCY                : 22.800
REMARK 200  R MERGE                    (I) : NULL
REMARK 200  R SYM                      (I) : 0.05200
REMARK 200  <I/SIGMA(I)> FOR THE DATA SET  : 21.3000
REMARK 200
REMARK 200 IN THE HIGHEST RESOLUTION SHELL.
REMARK 200  HIGHEST RESOLUTION SHELL, RANGE HIGH (A)  : 1.90
REMARK 200  HIGHEST RESOLUTION SHELL, RANGE LOW  (A)  : 1.97
REMARK 200  COMPLETENESS FOR SHELL     (%): 99.7
REMARK 200  DATA REDUNDANCY IN SHELL       : 22.80
REMARK 200  R MERGE FOR SHELL          (I) : NULL
REMARK 200  R SYM FOR SHELL            (I): 0.05200
REMARK 200  <I/SIGMA(I)> FOR SHELL         : NULL
REMARK 200
REMARK 200 DIFFRACTION PROTOCOL: SINGLE WAVELENGTH
REMARK 200 METHOD USED TO DETERMINE THE STRUCTURE: SAD
REMARK 200 SOFTWARE USED: SOLVE
REMARK 200 STARTING MODEL: NULL
REMARK 200
REMARK 200 REMARK: SF FILE CONTAINS FRIEDEL PAIRS.
REMARK 280
REMARK 280 CRYSTAL
REMARK 280 SOLVENT CONTENT, VS   (%): 61.08
REMARK 280 MATTHEWS COEFFICIENT, VM (ANGSTROMS**3/DA) : 3.16
REMARK 280
REMARK 280 CRYSTALLIZATION CONDITIONS: 200MM LI2S04, 100MM TRIS, 20%
PEG 5000
REMARK 280 MME, PH 8, VAPOR DIFFUSION, HANGING DROP, TEMPERATURE 289K
REMARK 290
REMARK 290 CRYSTALLOGRAPHIC SYMMETRY
REMARK 290 SYMMETRY OPERATORS FOR SPACE GROUP: P 62 2 2
REMARK 290
REMARK 290      SYMOP   SYMMETRY
REMARK 290     NNNMMM   OPERATOR
REMARK 290       1555   X,Y,Z
REMARK 290       2555   -Y,X-Y,Z+2/3
REMARK 290       3555   -X+Y, -X, Z+l/3
REMARK 290       4555   -X,-Y,Z
REMARK 290       5555   Y,-X+Y, Z+2/3
REMARK 290       6555   X-Y, X,Z+l/3
REMARK 290       7555   Y,X, -Z+2/3
REMARK 290       8555   X-Y,-Y,-Z
REMARK 290       9555   -X, -X+Y, -Z+l/3
REMARK 290      10555   -Y,-X, -Z+2/3
REMARK 290      11555   -X+Y,Y,-Z
REMARK 290      12555   X,X-Y, -Z+l/3
REMARK 290
REMARK 290     WHERE NNN -> OPERATOR NUMBER
```

```
REMARK 290            MMM -> TRANSLATION VECTOR
REMARK 290
REMARK 290 CRYSTALLOGRAPHIC SYMMETRY TRANSFORMATIONS
REMARK 290 THE FOLLOWING TRANSFORMATIONS OPERATE ON THE ATOM/HETATM
REMARK 290 RECORDS IN THIS ENTRY TO PRODUCE CRYSTALLOGRAPHICALLY
REMARK 290 RELATED MOLECULES.
REMARK 290   SMTRY1    1  1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2    1  0.000000  1.000000  0.000000        0.00000
REMARK 290   SMTRY3    1  0.000000  0.000000  1.000000        0.00000
REMARK 290   SMTRY1    2 -0.500000 -0.866025  0.000000        0.00000
REMARK 290   SMTRY2    2  0.866025 -0.500000  0.000000        0.00000
REMARK 290   SMTRY3    2  0.000000  0.000000  1.000000       80.69000
REMARK 290   SMTRY1    3 -0.500000  0.866025  0.000000        0.00000
REMARK 290   SMTRY2    3 -0.866025 -0.500000  0.000000        0.00000
REMARK 290   SMTRY3    3  0.000000  0.000000  1.000000       40.34500
REMARK 290   SMTRY1    4 -1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2    4  0.000000 -1.000000  0.000000        0.00000
REMARK 290   SMTRY3    4  0.000000  0.000000  1.000000        0.00000
REMARK 290   SMTRY1    5  0.500000  0.866025  0.000000        0.00000
REMARK 290   SMTRY2    5 -0.866025  0.500000  0.000000        0.00000
REMARK 290   SMTRY3    5  0.000000  0.000000  1.000000       80.69000
REMARK 290   SMTRY1    6  0.500000 -0.866025  0.000000        0.00000
REMARK 290   SMTRY2    6  0.866025  0.500000  0.000000        0.00000
REMARK 290   SMTRY3    6  0.000000  0.000000  1.000000       40.34500
REMARK 290   SMTRY1    7 -0.500000  0.866025  0.000000        0.00000
REMARK 290   SMTRY2    7  0.866025  0.500000  0.000000        0.00000
REMARK 290   SMTRY3    7  0.000000  0.000000 -1.000000       80.69000
REMARK 290   SMTRY1    8  1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2    8  0.000000 -1.000000  0.000000        0.00000
REMARK 290   SMTRY3    8  0.000000  0.000000 -1.000000        0.00000
REMARK 290   SMTRY1    9 -0.500000 -0.866025  0.000000        0.00000
REMARK 290   SMTRY2    9 -0.866025  0.500000  0.000000        0.00000
REMARK 290   SMTRY3    9  0.000000  0.000000 -1.000000       40.34500
REMARK 290   SMTRY1   10  0.500000 -0.866025  0.000000        0.00000
REMARK 290   SMTRY2   10 -0.866025 -0.500000  0.000000        0.00000
REMARK 290   SMTRY3   10  0.000000  0.000000 -1.000000       80.69000
REMARK 290   SMTRY1   11 -1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2   11  0.000000  1.000000  0.000000        0.00000
REMARK 290   SMTRY3   11  0.000000  0.000000 -1.000000        0.00000
REMARK 290   SMTRY1   12  0.500000  0.866025  0.000000        0.00000
REMARK 290   SMTRY2   12  0.866025 -0.500000  0.000000        0.00000
REMARK 290   SMTRY3   12  0.000000  0.000000 -1.000000       40.34500
REMARK 290
REMARK 290 REMARK: NULL
REMARK 300
REMARK 300 BIOMOLECULE:  1
REMARK 300 SEE REMARK 350 FOR THE AUTHOR PROVIDED AND/OR PROGRAM
REMARK 300 GENERATED ASSEMBLY INFORMATION FOR THE STRUCTURE IN
REMARK 300 THIS ENTRY. THE REMARK MAY ALSO PROVIDE INFORMATION ON
REMARK 300 BURIED SURFACE AREA.
REMARK 350
REMARK 350 COORDINATES FOR A COMPLETE MULTIMER REPRESENTING THE KNOWN
REMARK 350 BIOLOGICALLY SIGNIFICANT OLIGOMERI ZATION STATE OF THE
REMARK 350 MOLECULE CAN BE GENERATED BY APPLYING BIOMT TRANSFORMATIONS
REMARK 350 GIVEN BELOW.  BOTH NON-CRYSTALLOGRAPHIC  AND
REMARK 350 CRYSTALLOGRAPHIC  OPERATIONS  ARE GIVEN.
REMARK 350
REMARK 350 BIOMOLECULE:  1
REMARK 350 AUTHOR DETERMINED BIOLOGICAL UNIT: DIMERIC
```

```
REMARK 350 SOFTWARE DETERMINED QUATERNARY STRUCTURE: DIMERIC
REMARK 350 SOFTWARE USED: PISA
REMARK 350 TOTAL BURIED SURFACE AREA: 3680 ANGSTROM** 2
REMARK 350 SURFACE AREA OF THE COMPLEX: 12910 ANGSTROM* *2
REMARK 350 CHANGE IN SOLVENT FREE ENERGY: -116.0 KCAL/MOL
REMARK 350 APPLY THE FOLLOWING TO CHAINS: A
REMARK 350   BIOMT1    1  1.000000   0.000000   0.000000         0.00000
REMARK 350   BIOMT2    1  0.000000   1.000000   0.000000         0.00000
REMARK 350   BIOMT3    1  0.000000   0.000000   1.000000         0.00000
REMARK 350   BIOMT1    2  0.500000   0.866025   0.000000         0.00000
REMARK 350   BIOMT2    2  0.866025  -0.500000   0.000000         0.00000
REMARK 350   BIOMT3    2  0.000000   0.000000  -1.000000        40.34500
REMARK 375
REMARK 375 SPECIAL POSITION
REMARK 375 THE FOLLOWING ATOMS ARE FOUND TO BE WITHIN 0.15 ANGSTROMS
REMARK 375 OF A SYMMETRY RELATED ATOM AND ARE ASSUMED TO BE ON SPECIAL
REMARK 375 POSITIONS.
REMARK 375
REMARK 375 ATOM RES CSSEQI
REMARK 375      HOH A 163  LIES ON A SPECIAL POSITION.
REMARK 375      HOH A 138  LIES ON A SPECIAL POSITION.
REMARK 465
REMARK 465 MISSING RESIDUES
REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE.)
REMARK 465
REMARK 465   M RES C SSSEQI
REMARK "465     LEU A    97
REMARK 465     GLU A    98
REMARK 465     ASN A    99
REMARK 465     VAL A   100
REMARK 465     HIS A   101
REMARK 470
REMARK 470 MISSING ATOM
REMARK 470 THE FOLLOWING RESIDUES HAVE MISSING ATOMS (M=MODEL NUMBER;
REMARK 470 RES=RESIDUE NAME; C=CHAIN IDENTIFIER; SSEQ=SEQUENCE NUMBER;
REMARK 470 I=INSERTION CODE) :
REMARK 470   M RES CSSEQI  ATOMS
REMARK 470     ALA A  95    CB
REMARK 500
REMARK 500 GEOMETRY AND STEREOCHEMISTRY
REMARK 500 SUBTOPIC: TORSION ANGLES
REMARK 500
REMARK 500 TORSION ANGLES OUTSIDE THE EXPECTED RAMACHANDRAN REGIONS:
REMARK 500 (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN IDENTIFIER;
REMARK 500 SSEQ=SEQUENCE NUMBER; I=INSERTION CODE) .
REMARK 500
REMARK 500 STANDARD TABLE:
REMARK 500 FORMAT :(10X, I3,IX, A3, IX, Al,14,Al,4X, F7.2,3X, F7.2)
REMARK 500
REMARK 500 EXPECTED VALUES: GJ KLEYWEGT AND TA JONES (1996). PHI/PSI-
REMARK 500 CHOLOGY: RAMACHANDRAN REVISITED. STRUCTURE 4, 1395 - 1400
REMARK 500
REMARK 500  M RES CSSEQI         PSI        PHI
REMARK 500    ALA A  95       53.06     -55.19
REMARK 500
REMARK 500 REMARK: NULL
REMARK 620
```

```
REMARK- 620 METAL COORDINATION-
REMARK  620 (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN IDENTIFIER;
REMARK  620 SSEQ=SEQUENCE NUMBER; I=INSERTION CODE) :
REMARK  620
REMARK  620 COORDINATION ANGLES FOR: M RES CSSEQI METAL
REMARK  620                          ZN A 104 ZN
REMARK  620 N RES CSSEQI ATOM
REMARK  620 1 HIS A 22 ND1
REMARK  620 2 CYS A 40 SG 117.5
REMARK- 620 3 CYS A 20 SG 103..9 105.8
REMARK  620 4 CYS A 43 SG 107..3 108.9 113.5
REMARK  620 N                1    2    3
REMARK  620
REMARK  620 COORDINATION ANGLES FOR: M RES CSSEQI METAL
REMARK  620                          ZN A 102 ZN
REMARK  620 N RES CSSEQI ATOM
REMARK  620 1 HIS A 80 NE2
REMARK  620 2 HIS A 85 NE2 101.8
REMARK  620 3 CYS A 67 SG 107.5 116.8
REMARK  620 N                1    2
REMARK  620
REMARK  620 COORDINATION ANGLES FOR: M RES CSSEQI METAL
REMARK  620                          ZN A 103 ZN
REMARK  620 N RES CSSEQI ATOM
REMARK  620 1 CYS A 7 SG
REMARK  620 2 CYS A' 25 SG 112.9
REMARK  620 3 CYS A 4 SG 104.5 107.2
REMARK  620 4 CYS A 28 SG 111.0 110.3 110.8
REMARK  620 N                1    2    3
REMARK  800
REMARK  800 SITE
REMARK  800 SITE_IDENTIFIER: AC1
REMARK  8.00 EVIDENCE_CODE: SOFTWARE
REMARK  800 SITE_DESCRIPTION: BINDING SITE FOR RESIDUE ZN A 102
REMARK  800
REMARK  800 SITE_IDENTIFIER: AC2
REMARK  800 EVIDENCE_CODE: SOFTWARE
REMARK  800 SITE_DESCRIPTION: .BINDING SITE FOR RESIDUE ZN A 103
REMARK  800
REMARK  800 SITE_IDENTIFIER: AC3
REMARK  800 EVIDENCE_CODE: SOFTWARE
REMARK  800 SITE_DESCRIPTION: BINDING SITE FOR RESIDUE ZN A 104
DBREF   3VK6 A    1   101 UNP    Q9JIY2   HAKAI_MOUSE    106    206
SEQRES   1 A  101  VAL HIS PHE CYS ASP LYS CYS GLY LEU PRO ILE LYS VAL
SEQRES   2 A  101  TYR GLY ARG MET ILE PRO CYS LYS HIS VAL PHE CYS TYR
SEQRES   3 A  101  ASP CYS ALA ILE LEU HIS GLU LYS LYS GLY ASP LYS MET
SEQRES   4 A  101  CYS PRO GLY CYS SER ASP PRO VAL GLN ARG ILE GLU GLN
SEQRES   5 A  101  CYS THR ARG GLY SER LEU PHE MET CYS SER ILE VAL GLN
SEQRES   6 A  101  GLY CYS LYS ARG THR TYR LEU SER GLN ARG ASP LEU GLN
SEQRES   7 A  101  ALA HIS ILE ASN HIS ARG HIS MET ARG ALA GLY LYS PRO
SEQRES   8 A  101  VAL THR ARG ALA SER LEU GLU ASN VAL HIS
HET     ZN  A 102       1
HET     ZN  A 103       1
HET     ZN  A 104       1
HETNAM      ZN ZINC ION
FORMUL   2   ZN    3(ZN 2+)
FORMUL   5  HOH   *70(H2 O)
HELIX    1   1 TYR A   26 LYS A   35 1
```

```
10
HELIX    2   2 GLY A   56  LEU A   58  5
3
HELIX    3   3 SER A   73  HIS A   85  1
13
SHEET    1   A 3 VAL A  23  CYS A  25  0
SHEET    2   A 3 VAL A  13  ILE A  18 -1  N  GLY A  15   O  PHE A  24
SHEET    3   A 3 ARG A  49  THR A  54 -1  O  CYS A  53   N  TYR A  14
LINK         ND1 HIS A  22                ZN    ZN A 104      1555
1555  1..98
LINK         NE2 HIS A  80                ZN    ZN A 102      1555
1555  2..08
LINK         NE2 HIS A  85                ZN    ZN A 102      1555
1555  2..12
LINK         SG  CYS A  40                ZN    ZN A 104      1555
1555  2..29
LINK         SG  CYS A  20                ZN    ZN A 104      1555
1555  2..32
LINK         SG  CYS A  67                ZN    ZN A 102      1555
1555  2..33
LINK         SG  CYS A   7                ZN    ZN A 103      1555
1555  2..33
LINK         SG  CYS A  25                ZN    ZN A 103      1555
1555  2..35
LINK         SG  CYS A  43                ZN    ZN A 104      1555
1555  2..36
LINK         SG  CYS A   4                ZN    ZN A 103      1555
1555  2..38
LINK         SG  CYS A  28                ZN    ZN A 103      1555
1555  2.39
CISPEP   1 ILE A   18    PRO A   19          0         9.66
SITE     1 AC1   4 CYS A  61  CYS A  67  HIS A  80  HIS A  85
SITE     1 AC2   4 CYS A   4  CYS A   7  CYS A  25  CYS A  28
SITE     1 AC3   4 CYS A  20  HIS A  22  CYS A  40  CYS A  43
CRYST1   64.657   64.657  121.035  90.00  90.00 120.00 P 62 2 2    12
0RIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.015466  0.008929  0.000000        0.00000
SCALE2      0.000000  0.017859  0.000000        0.00000
SCALE3      0.000000  0.000000  0.008262        0.00000
ATOM      1  N   VAL A   1     -14.217 -31.908  31.023  1.00 43.24
N
ATOM      2  CA  VAL A   1     --13.680 -32.592  29.844  1.00 38.28
C
ATOM      3  C   VAL A   1     --13.920 -31.761  28.590  1.00 39.47
C  .
ATOM      4  O   VAL A   1     --15.044 -31.691  28.097  1.00 45.65
0
ATOM      5  CB  VAL A   1     --14.318 -34.009  29.682  1.00 42.95
C
ATOM      6  CGI VAL A   1     --13.873 -34.673  28.399  1.00 40.15
C
ATOM      7  CG2 VAL A   1     --13.966 -34.909  30.880  1.00 42.58
C
ATOM      8  N   HIS A   2     --12.866 -31.123  28.088  1.00 34.05
N
ATOM      9  CA  HIS A   2     --12.914 -30.352  26.861  0.98 37.02
```

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C
ATOM      10  C   HIS A   2      -11.919 -30.960  25.866   1.00 34.85
C
ATOM      11  0   HIS A   2      -10.717 -31.057  26.161   1.00 32.70
0
ATOM      12  CB  HIS A   2      -12.537 -28.897  27.149   1.00 36.14
C
ATOM      13  CG  HIS A   2      -12.569 -28.009  25.947   0.64 37.61
C
ATOM      14  ND1 HIS A   2      -13.311 -28.301  24.823   0.20 37.81
N
ATOM      15  CD2 HIS A   2      -11.962 -26.823  25.700   0.30 38.48
C
ATOM      16  CE1 HIS A   2      -13.151 -27.339  23.931   0.76 39.37
C
ATOM      17  NE2 HIS A   2      -12.341 -26.428  24.440   0.38 39.67
N
ATOM      18  N   PHE A   3      -12.411 -31.379  24.702   1.00 32.80
N
ATOM      19  CA  PHE A   3      -11.522 -31.929  23.678   1.00 32.28
C
ATOM      20  C   PHE A   3      -10.999 -30.855  22.739   1.00 35.87
C
ATOM      21  O   PHE A   3      -11.754 -29.978  22.295   1.00 34.57
0
ATOM      22  CB  PHE A   3      -12.233 -33.040  22.888   1.00 33.26
C
ATOM      23  CG  PHE A   3      -12.316 -34.336  23.622   1.00 33.39
C
ATOM      24  CD1 PHE A   3      -13.262 -34.525  24.623   1.00 35.97
C
ATOM      25  CD2 PHE A   3      -11.451 -35.372  23.326   1.00 32.57
C
ATOM      26  CE1 PHE A   3      -13.334 -35.723  25.305   1.00 35.87
C
ATOM      27  CE2 PHE A   3      -11.524 -36.571  24.015   1.00 31.51
C
ATOM      28  CZ  PHE A   3      -12.467 -36.746  24.994   1.00 35.56
C
ATOM      29  N   CYS A   4       -9.710 -30.929  22.426   1.00 32.09
N
ATOM      30  CA  CYS A   4       -9.109 -30.014  21.473   1.00 32.02
C
ATOM      31  C   CYS A   4       -9.560 -30.347  20.055   1.00 35.66
C
ATOM      32  O   CYS A   4       -9.502 -31.506  19.642   1.00 32.63
0
ATOM      33  CB  CYS A   4       -7.591 -30.073  21.550   1.00 31.91
C
ATOM      34  SG  CYS A   4       -6.800 -28.933  20.409   1.00 31.07
S
ATOM      35  N   ASP A   5      -10.005 -29.334  19.310   1.00 35.12
N
ATOM      36  CA  ASP A   5      -10.470 -29.558  17.937   1.00 35.99
C
ATOM      37  C   ASP A   5       -9.344 -29.551  16.921   1.00 38.96
C
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ATOM     38  O   ASP A   5      -9.578 -29.737  15.724  1.00 37.64
O
ATOM     39  CB  ASP A   5     -11.572 -28.568  17.529  1.00 41.25
C
ATOM     40  CG  ASP A   5     -11.108 -27.117  17.538  1.00 43.64
C
ATOM     41  GDI ASP A   5      -9.894 -26.855  17.668  1.00 41.44
O
ATOM     42  OD2 ASP A   5     -11.981 -26.227  17.403  1.00 50.17
O
ATOM     43  N   LYS A   6      -8.123 -29.340  17.398  1.00 36.54
N
ATOM     44  CA  LYS A   6      -6.946 -29.420  16.542  1.00 37.90
C
ATOM     45  C   LYS A   6      -6.322 -30.798  16.637  1.00 39.23
C
ATOM     46  O   LYS A   6      -6.194 -31.513  15.637  1.00 37.67
O
ATOM     47  CB  LYS A   6      -5.901 -28.389  16.971  1.00 37.88
C
ATOM     48  CG  LYS A   6      -5.696 -27.224  16.022  1.00 46.51
C
ATOM     49  CD  LYS A   6      -6.967 -26.414  15.818  1.00 47.95
C
ATOM     50  CE  LYS A   6      -7.667 -26.817  14.525  1.00 48.63
C
ATOM     51  NZ  LYS A   6      -6.673 -27.052  13.439  1.00 49.54
N
ATOM     52  N   CYS A   7      -5.921 -31.164  17.850  1.00 34.08
N
ATOM     53  CA  CYS A   7      -5.224 -32.427  18.071  1.00 34.54
C
ATOM     54  C   CYS A   7      -6.175 -33.574  18.410  1.00 35.78
C
ATOM     55  O   CYS A   7      -5.827 -34.734  18.246  1.00 37.45
O
ATOM     56  CB  CYS A   7      -4.139 -32.280  19.151  1.00 33.57
C
ATOM     57  SG  CYS A   7      -4.745 -32.011  20.856  1.00 31.74
S
ATOM     58  N   GLY A   8      -7.367 -33.251  18.901  1.00 31.74
N
ATOM     59  CA  GLY A   8      -8.353 -34.265  19.226  1.00 31.07
C
ATOM     60  C   GLY A   8      -8.282 -34.867  20.616  1.00 31.77
C
ATOM     61  O   GLY A   8      -9.075 -35.742  20.951  1.00 33.42
O
ATOM     62  N   LEU A   9      -7.351 -34.397  21.441  1.00 27.25
N
ATOM     63  CA  LEU A   9      -7.193 -34.943  22.779  1.00 28.34
C
ATOM     64  C   LEU A   9      -7.833 -34.036  23.842  1.00 31.76
C
ATOM     65  O   LEU A   9      -7.957 -32.820  23.643  1.00 27.98
O
ATOM     66  CB  LEU A   9      -5.723 -35.158  23.107  1.00 29.93
C
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ATOM 67 CG LEU A 9 −4.938 -36.179 22.267 1.00 29.89
C
ATOM 68 CD1 LEU A 9 −3.457 -36.036 22.586 1.00 31.72
C
ATOM 69 CD2 LEU A 9 −5.408 -37.611 22.531 1.00 28.30
C
ATOM 70 N PRO A 10 −8.250 -34.640 24.964 1.00 31.04
N
ATOM 71 CA PRO A 10 −8.829 -33.883 26.078 1.00 32.07
C
ATOM 72 C PRO A 10 −7.798 -32.927 26.647 1.00 34.78
C
ATOM 73 O PRO A 10 −6.625 -33.283 26.803 1.00 33.68
0
ATOM 74 CB PRO A 10 −9.221 -34.965 27.092 1.00 35.67
C
ATOM 75 CG PRO A 10 −8.466 -36.187 26.703 1.00 31.83
C
ATOM 76 CD PRO A 10 −8.201 -36.089 25.225 1.00 32.04
C
ATOM 77 N ILE A 11 −8.223 -31.699 26.919 1.00 31.68
N
ATOM 78 CA ILE A 11 −7.300 -30.668 27.362 1.00 31.74
C
ATOM 79 C ILE A 11 −7.214 -30.674 28.888 1.00 34.92
C
ATOM 80 O ILE A 11 −8.236 -30.642 29.584 1.00 34.62
0
ATOM 81 CB ILE A 11 −7.750 -29.276 26,884 1.00 32.66
C
ATOM 82 CGI ILE A 11 −7.891 -29.260 25.355 1.00 30.36
C
ATOM 83 CG2 ILE A 11 −6.774 -28.194 27.351 1.00 32.29
C
ATOM 84 CD1 ILE A 11 −8.789 -28.127 24.849 1.00 34.02
C
ATOM 85 N LYS A 12 −5.992 -30.728 29.397 1.00 34.77
N
ATOM 86 CA LYS A 12 −5.780 -30.600 30.832 1.00 39.37
C
ATOM 87 C LYS A 12 −5.033 -29.313 31.165 1.00 37.98
C
ATOM 88 O LYS A 12 −5.190 -28.761 32.262 1.00 39.91
0
ATOM 89 CB LYS A 12 −5.066 -31.832 31.402 1.00 45.11
C
ATOM 90 CG LYS A 12 −3.700 -32.098 30.818 1.00 43.92
C
ATOM 91 CD LYS A 12 −3.607 -33.512 30.238 1.00 50.37
C
ATOM 92 CE LYS A 12 −2.198 -33.790 29.715 1.00 48.20
C
ATOM 93 NZ LYS A 12 −1.625 -32.564 29.075- 1.00 48.39
N
ATOM 94 N VAL A 13 −4.236 -28.826 30.222 1.00 33.60
N
ATOM 95 CA VAL A 13 −3.549 -27.553 30.399 1.00 35.83
C

ATOM 96 C VAL A 13 −3.859 -26. 618 29.238 1.00 35.15
C
.ATOM 97 0 VAL A 13 −3.737 -27 .019 28.083 1.00 32.14
0
ATOM 98 CB VAL A 13 −2 .019 -27.736 30.490 1.00 37.74
C
ATOM 99 CGI VAL A 13 −1.327 -26. 386 30. 600 1.00 36.88
C
ATOM 100 CG2 VAL A 13 −1 .657 -28 .615 31. 690 1.00 40.88
C
ATOM 101 N TYR A 14 −4.239 -25.377 29.541 1.00 34.26
N
ATOM 102 CA TYR- A 14 −4 .574 -24 .398 28 .495 1.00 .34.13
C
ATOM 103 C TYR A 14 −3.478 -23. 392 28.262 1.00 33.08
C
ATOM 104 O TYR A 14 −2.719 -23.0 66 29.170 1.00 33.96
0
ATOM 105 CB TYR A 14 −5.839 -23. 608 28.849 1.00 31.93
C
ATOM 106 CG TYR A 14 −7 .105 -24 .402 28.787 1.00 33.92
C
ATOM 107 CD1 TYR A 14 −7 .898 -24 .396 27 .636 1.00 33.65
C
ATOM 108 CD2 TYR A 14 −7.518 -25.148 29. 866 1.00 36.49
C
ATOM 109 CE1 TYR A 14 −9.063 -25. 126 27.583 1.00 36.26
C
ATOM 110 CE2 TYR A 14 −8 .667 -25.874 29. 822 1.00 40.39
C .
ATOM 111 CZ TYR A 14 −9.435 -25.866 28 .680 1.00 37.92
C
ATOM 112 OH TYR A 14 −10.591 -26. 602 28 .660 1.00 45.77
O
ATOM 113 N GLY A 15 −3.417 -22.880 27 .035 1...00 34.07
N
ATOM 114 CA GLY A 15 −2 .627 -21.704 26.737 1.00 33.20
C
ATOM 115 C GLY A 15 −3.575 -20.518 26. 672 1.00 32.07
C
ATOM 116 O GLY A 15 −4 .593 -20.571 25.974 1.00 33.11
0
ATOM 117 N ARG A 16 −3.271 -19. 468 27 .423 1,00 32.41
N
ATOM 118 CA ARG A 16 −4 .098 -18 .265 27.432 1.00 29.19
C
ATOM 119 C ARG A 16 −3.351 -17.200 26. 646 1.0,0 33.70
C
ATOM 120 O ARG A 16 −2.185 -16. 905 26. 933 1.00 33.66
0
ATOM 121 CB ARG A 16 −4 .380 -17 .818 28 .863 1.00 33.11
C
ATOM 122 CG ARG A 16 −5.149 -16.514 29.013 1.00 29.75
C
ATOM 123 CD ARG A 16 −5 .644 -16. 415 30.460 1.00 32.31
C
ATOM 124 NE ARG A 16 −6 .408 -15.202 30.765 1.00 31.60
N

ATOM 125 .CZ ARG A 16 −5.905 -14.131 31.373 1.00 33.56
C
ATOM 126 NH1 ARG A 16 −4.625 -14.105 31.726 1.00 33.52
N
ATOM 127 NH2 ARG A 16 −6.683 -13.080 31.627 1.00 30.00
N
ATOM 128 N MET A 17 −3.993 -16.675 25.605 1.00 30.01
N
ATOM 129 CA MET A 17 −3.333 -15.700 24.758 1.00 29.69
C
ATOM 130 C MET A 17 −3.315 -14.355 25.468 1.00 32.77
C
ATOM 131 O MET A 17 −4.326 -13.924 26.014 1.00 34.13
0
ATOM 132 CB MET A 17 −4.073 -15.569 23.410 1.00 31.74
C
ATOM 133 CG MET A 17 −4.284 -16.884 22.634 1.00 30.41
C
ATOM 134 SD MET A 17 −2.796 -17.730 22.031 1.00 30.02
S
ATOM 135 CE MET A 17 −2.728 -18.978 23.313 1.00 33.13
C
ATOM 136 N ILE A 18 −2.174 -13.682 25.461 1.00 33.95
N
ATOM 137 CA ILE A 18 −2.129 -12.317 25.961 1.00 36.17
C
ATOM 138 C ILE A 18 −1.858 -11.383 24.774 1.00 37.58
C
ATOM 139 0 ILE A 18 −0.904 -11.614 24.032 1.00 37.31-
0
ATOM 140 CB ILE A 18 −1.041 -12.163 27.041 1.00 37.48
C
ATOM 141 CGI ILE A 18 −1.304 -13.127 28.214 1.00 36.97
C
ATOM 142 CG2 ILE A 18 −0.929 -10.717 27.512 1.00 40.45
C
ATOM 143 CD1 ILE A 18 −2.556 -12.814 29.034 1.00 37.15
C
ATOM 144 N PRO A 19 −2.657 -10.297 24.624 1.00 36.04
N
ATOM 145 CA PRO A 19 −3.649 -9.786 25.581 1.00 34.39
C
ATOM 146 C PRO A 19 −5.128 -10.060 25.277 I.00 36.76
C
ATOM 147 O PRO A 19 −5.988 -9.586 26.035 1.00 37.23
0
ATOM 148 CB PRO A 19 −3.401 -8.276 25.533 1.00 41.24
C
ATOM 149 CG PRO A 19 −2.984 -8.020 24.101 1.00 38.16
C
ATOM 150 CD PRO A 19 −2.380 -9.311 23.561 1.00 39.06
C
ATOM 151 N CYS A 20 −5.437 -10.817 24.230 1.00 33.89
N
ATOM 152 CA CYS A 20 −6.840 -11.018 23.866 1.00 35.21
C
ATOM 153 C CYS A 20 −7.598 -11.950 24.829 1.00 33.91
C

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ATOM    154  0   GYS A  20      -8.834  -11. 931  24 .875  1.00  31.07
O
ATOM    155  CB  CYS A  20      -6. 960 -11. 494  22.416   1.00  33.97
C
ATOM    156  SG  CYS A  20      -6. 504 -13,233   22. 179  1.00  33.10
S
ATOM    157  N   LYS A  21      -6.845  -12 .756  25.584   1.00  29.78
N
ATOM    158  CA  LYS A  21      -7.359  -13. 601  26. 673  1.00  30.24
C
ATOM    159  C . LYS A  21      -8 .178 -14 .830  26.280   1.00  28.76
C
ATOM    160  O   LYS A  21      -8.805  -15. 463  27. 142  1.00  27.96
0
ATOM    161  CB  LYS A  21      -8 .105 -12.768   27 .722  1.00  28.56
C
ATOM    162  CG  LYS A  21      -7 .219 -11.732   28 .417  1.00  32.15
C
ATOM    163  CD  LYS A  21      -8.070  -10.813  29. 302  1.00  33.23
C
ATOM    164  CE  LYS A  21      -7 .292   -9. 571  29.723  1.00  37.90
C
ATOM    165  NZ  LYS A  21      -6.807    -8 .785  28 .560  1.00  38.35
N
ATOM    166  N   HIS A  22      -8.181  -15. 164  24 .996  1.00  27.92
N .
ATOM    167  CA  HIS A  22      -8 .771 -16.428   24 .551  1.00  27.15
C
ATOM    168  C   HIS A  22      -7 .908 -17. 626  24 .979  1.00  30.80
C
ATOM    169  O   HIS A  22      -6. 697 -17.481   25.177   1.00  30.10
O
ATOM    170  CB  HIS A  22      -8.999  -16. 415  23.046   1.00  29.51
C
ATOM    171  CG  HIS A  22      -9. 983 -15. 372  22. 615  1.00  30.39
C
ATOM    172  ND1 HIS A  22      -9. 667 -14 .373  21.717   1.00  30.28
N
ATOM    173  CD2 HIS A  22     --11.267 -15. 152  22. 988  1.00  30.99
C
ATOM    174  CE1 HIS A  22     --10.719 -13. 585  21.550   1.00  30.62
C
ATOM    175  NE2 HIS A  22     --11.-705 -14 .037  22.300   1.00  29.25
N
ATOM    176  N   VAL A  23      -8 .522 -18 .795  25.139   1.00  29.80
N
ATOM    177  CA  VAL A  23      -7.745  -19. 968  25.549   1.00  30.93
C
ATOM    178  C   VAL A  23      -7.891  -21. 135  24 .590  1.00  33.12
C
ATOM    179  O   VAL A  23      -8 .924 -21.304   23. 914  1.00  29.29
0
ATOM    180  CB  VAL A  23      -8.066  -20. 441  26. 987  1.00  29.60
C
ATOM    181  CGI VAL A  23      -7.597  -19. 396  28 .018  1.00  31.89
C
ATOM    182  CG2 VAL A  23      -9.542  -20.760   27 .128  1.00  31.43
C
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ATOM    183  N   PHE A  24      -6.837 -21.940  24.536  1.00 30.81
N
ATOM    184  CA  PHE A  24      -6.787 -23.063  23.609  1.00 32.98
C
ATOM    185  C   PHE A  24      -5.949 -24.168  24.244  1.00 34.89
C
ATOM    186  O   PHE A  24      -5.245 -23.931  25.215  1.00 32.00
O
ATOM    187  CB  PHE A  24      -6.151 -22.618  22.295  1.00 33.83
C
ATOM    188  CG  PHE A  24      -6.855 -21.446  21.658  1.00 34.75
C
ATOM    189  CD1 PHE A  24      -7.930 -21.652  20.808  1.00 34.89
C
ATOM    190  CD2 PHE A  24      -6.467 -20.148  21.950  1.00 34.03
C
ATOM    191  CE1 PHE A  24      -8.602 -20.580  20.243  1.00 36.57
C
ATOM    192  CE2 PHE A  24      -7.137 -19.059  21.379  1.00 34.37
C
ATOM    193  CZ  PHE A  24      -8.205 -19.286  20.532  1.00 33.08
C
ATOM    194  N   CYS A  25      -6.041 -25.366  23.685  1.00 33.09
N
ATOM    195  CA  CYS A  25      -5.190 -26.478  24.075  1.00 31.38
C
ATOM    196  C   CYS A  25      -3.748 -25.974  24.184  1.00 32.44
C
ATOM    197  O   CYS A  25      -3.233 -25.376  23.252  1.00 32.95
O
ATOM    198  CB  CYS A  25      -5.309 -27.549  22.998  1.00 31.93
C
ATOM    199  SG  CYS A  25      -4.058 -28.857  23.054  1.00 32.02
S
ATOM    200  N   TYR A  26      -3.098 -26.187  25.326  1.00 33.66
N
ATOM    201  CA  TYR A  26      -1.759 -25.617  25.515  1.00 35.02
C
ATOM    202  C   TYR A  26      -0.730 -26.142  24.505  1.00 37.39
C
ATOM    203  O   TYR A  26       0.067 -25.376  23.950  1.00 35.25
O
ATOM    204  CB  TYR A  26      -1.251 -25.843  26.943  1.00 37.34
C
ATOM    205  CG  TYR A  26       0.099 -25.208  27.180  1.00 37.27
C
ATOM    206  CD1 TYR A  26       0.221 -23.835  27.302  1.00 37.64
C
ATOM    207  CD2 TYR A  26       1.252 -25.981  27.267  1.00 41.59
C
ATOM    208  CE1 TYR A  26       1.444 -23.242  27.511  1.00 41.92
C
ATOM    209  CE2 TYR A  26       2.484 -25.397  27.469  1.00 42.21
C
ATOM    210  CZ  TYR A  26       2.574 -24.023  27.588  1.00 43.26
C
ATOM    211  OH  TYR A  26       3.792 -23.415  27.799  1.00 48.53
O
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ATOM    212  N   ASP A  27      -0.740 -27.453  24.280  1.00 35.01
N
ATOM    213  CA  ASP A  27       0.244 -28.080  23.405  1.00 38.59
C
ATOM    214  C   ASP A  27       0.126 -27.552  21.973  1.00 38.88
C
ATOM    215  0   ASP A  27       1.127 -27.240  21.320  1.00 40.15
O
ATOM    216  CB  ASP A  27       0.095 -29.603  23.460  1.00 38.84
C
ATOM    217  CG  ASP A  27       0.478 -30.161  24.813  1.00 42.03
C
ATOM    218  OD1 ASP A  27       1.639 -29.978  25.203  1.00 45.14
0
ATOM    219  OD2 ASP A  27      -0.374 -30.757  25.503  1.00 45.01
O
ATOM    220  N   CYS A  28      -1.104 -27.410  21.500  1.00 36.98
N
ATOM    221  CA  CYS A  28      -1.320 -26.867  20.167  1.00 38.40
C
ATOM    222  C   CYS A  28      -0.965 -25.384  20.063  1.00 41.43
C
ATOM    223  0   CYS A  28      -0.406 -24.945  19.056  1.00 45.87
0
ATOM    224  CB  CYS A  28      -2.746 -27.135  19.695  1.00 34.77
C
ATOM    225  SG  CYS A  28      -3.035 -28.883  19.302  1.00 35.70
S
ATOM    226  N   ALA A  29      -1.289 -24.615  21.098  1.00 38.78
N
ATOM    227  CA  ALA A  29      -0.875 -23.218  21.160  1.00 39.90
C
ATOM    228  C   ALA A  29       0.650 -23.096  21.014  1.00 45.54
C
ATOM    229  0   ALA A  29       1.139 -22.325  20.188  1.00 47.21
0
ATOM    230  CB  ALA A  29      -1.353 -22.575  22.460  1.00 37.71
C
ATOM    231  N   ILE A  30       1.397 -23.860  21.809  1.00 45.46
N
ATOM    232  CA  ILE A  30       2.859 -23.828  21.732  1.00 45.69
C
ATOM    233  C   ILE A  30       3.340 -24.250  20.337  1.00 51.05
C
ATOM    234  O   ILE A  30       4.131 -23.558  19.697  1.00 53.45
0
ATOM    235  CB  ILE A  30       3.508 -24.733  22.804  1.00 45.45
C
ATOM    236  CGI ILE A  30       3.182 -24.228  24.211  1.00 46.94
C
ATOM    237  CG2 ILE A  30       5.012 -24.816  22.605  1.00 51.64
C
ATOM    238  CD1 ILE A  30       3.496 -22.761  24.437  1.00 46.56
C
ATOM    239  N   LEU A  31       2.847 -25.389  19.868  1.00 49.30
N
ATOM    240  CA  LEU A  31       3.188 -25.887  18.546  1.00 51.97
C
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ATOM    241  C   LEU A  31       3.024  -24.789  17.501  1.00  55.25
C
ATOM    242- 0.  LEU A  31       3.919  -24.543  16.689  1.00  58.11
0
ATOM    243  CB  LEU A  31       2.300  -27.086  18.196  1.00  50.87
C
ATOM    244  CG  LEU A  31       2.633  -27.802  16.891  1.00  53.86
C
ATOM    245  CD1 LEU A  31       3.997  -28.479  17.002  1.00  53.57
C
ATOM    246  CD2 LEU A  31       1.552  -28.812  16.541  1.00  53.40
C
ATOM    247  N   HIS A  32       1.865  -24.139  17.535  1.00  52.60
N
ATOM    248  CA  HIS A  32       1.534  -23.028  16.650  1.00  56.03
C
ATOM    249  C   HIS A  32       2.584  -21.915  16.792  1.00  56.91
C
ATOM    250  O   HIS A  32       3.056  -21.360  15.800  1.00  57.85
0
ATOM    251  CB  HIS A  32       0.136  -22.516  17.016  1.00  53.15
C
ATOM    252  CG  HIS A  32      --0.545  -21.731  15.938  1.00  59.33
C
ATOM    253  ND1 HIS A  32      --1.025  -20.454  16.140  1.00  59.34
N
ATOM    254 . CD2 HIS A  32      --0.857  -22.051  14.658  1.00  62.21
C
ATOM    255  CE1 HIS A  32      --1.590  -20.014  15.029  1.00  56.20
C
ATOM    256  NE2 HIS A  32      --1.501  -20.964  14.114  1.00  63.21
N
ATOM    257  N   GLU A  33       2.950  -21.606  18.035  1.00  54.43
N
ATOM    258  CA  GLU A  33       3.949  -20.580  18.329  1.00  55.34
C
ATOM    259  C   GLU A  33       5.322  -20.956  17.767  1.00  58.50
C
ATOM    260  0   GLU A  33       6.002  -20.128  17.158  1.00  57.87
0
ATOM    2-61 CB  GLU A  33       4.038  -20.352  19.839  1.00  54.98
C
ATOM    262  CG  GLU A  33       5.090  -19.351  20.282  1.00  52.81
C
ATOM    263  CD  GLU A  33       5.094  -19.153  21.786  1.00  53.52
C
ATOM    264  OE1 GLU A  33       5.323  -20.138  22.521  1.00  54.05
0
ATOM    265  OE2 GLU A  33       4.869  -18.012  22.240  1.00  52.87
0
ATOM    266  N   LYS A  34       5.712  -22.213  17.971  1.00  57.95
N
ATOM    267  CA  LYS A  34       6.956  -22.746  17.423  1.00  59.98
C
ATOM    268  C   LYS A  34       7.040  -22.564  15.908  1.00  61.96
C
ATOM    269  0   LYS A  34       8.125  -22.392  15.353  1.00  63.76
0
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ATOM    270  CB  LYS A  34       7.107 -24.233  17.769  1.00 58.57
C
ATOM    271  CG .LYS A  34       8.312 -24.889  17.105  1.00 64.23
C -
ATOM    272  CD  LYS A  34       8.291 -26.412  17.221  1.00 66,49
C
ATOM    273  CE  LYS A  34       9.352 -27.042  16.315  1.00 67.32
C
ATOM    274  NZ  LYS A  34       9.274 -28.534  16.270  1.00 66.12
N
ATOM    275  N   LYS A  35       5.891 -22.603  15.243  1.00 61.93
N
ATOM    276  CA  LYS A  35       5.841 -22.520  13.790  1.00 60.26
C
ATOM    277  C   LYS A  35       5.967 -21.084  13.286  1.00 61.88
C
ATOM    278  O   LYS A  35       6.106 -20.851  12.086  1.00 63.28
0
ATOM    279  CB  LYS A  35       4.539 -23.137  13.287  1.00 61.91
C
ATOM    280  CG  LYS A  35       4.479 -23.386  11.795  1.00 65.10
C
ATOM    281  CD  LYS A  35       3.206 -24.133  11.449  1.00 66.63
C
ATOM    282  CE  LYS A  35       2.878 -25.173  12.524  1.00 66.79
C
ATOM-   283  NZ  LYS A  35       3.998 -26.131  12.772  1.00 67.01
N
ATOM    284  N   GLY A  36       5.908 -20.125  14.204  1.00 61.73
N
ATOM    285  CA  GLY A  36       6.056 -18.722  13.854  1.00 62.76
C
ATOM    286  C   GLY A  36       4.758 -17.930  13.844  1.00 61.41
C
ATOM    287  O   GLY A  36       4.731 -16.747  13.479  1.00 60.53
0
ATOM    288  N   ASP A  37       3.672 -18.574  14.253  1.00 59.63
N
ATOM    289  CA  ASP A  37       2.375 -17.916  14.231  1.00 56.51
C
ATOM    290  C   ASP A  37       2.220 -16.999  15.437  1.00 54.20
C
ATOM    291  O   ASP A  37       2.408 -17.419  16.576  1.00 55.12
0
ATOM    292  CB  ASP A  37       1.252 -18.950  14.151  1.00 58.37
C
ATOM    293  CG  ASP A  37       1.321 -19.774  12.878  1.00 62.78
C
ATOM    294  OD1 ASP A  37       2.199 -19.479  12.032  1.00 64.75
0
ATOM    295  OD2 ASP A  37       0.510 -20.712  12.718  1.00 63.46
0
ATOM    296  N   LYS A  38       1.902 -15.736  15.178  1.00 49.95
N
ATOM    297  CA  LYS A  38       1.803 -14.753  16.245  1.00 47.72
C
ATOM    298  C   LYS A  38       0.400 -14.173  16.347  1.00 41.91
C
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ATOM    299  O   LYS A  38       0.186 -13.133  16.959  1.00 45.45
O
ATOM    300  CB  LYS A  38       2.842 -13.647  16.059  1.00 53.14
C
ATOM    301  CG  LYS A  38       4.278 -14.163  16.040  1.00 55.05
C
ATOM    302  CD  LYS A  38       5.264 -13.042  16.325  1.00 57.30
C
ATOM    303  CE  LYS A  38       6.682 -13.571  16.445  1.00 59.98
C
ATOM    304  NZ  LYS A  38       7.643 -12.484  16.795  1.00 56.91
N
ATOM    305  N   MET A  39      -0.556 -14.864  15.747  1.00 41.93
N
ATOM    306  CA  MET A  39      -1.946 -14.440  15.803  0.66 41.24
C
ATOM    307  C   MET A  39      -2.768 -15.455  16.592  0.74 39.91
C
ATOM    308  O   MET A  39      -2.489 -16.656  16.556  1.00 39.24
O
ATOM    309  CB  MET A  39      -2.506 -14.249  14.385  1.00 40.08
C
ATOM    310  CG  MET A  39      -2.063 -12.946  13.710  1.00 43.42
C
ATOM    311  SD  MET A  39      -2.931 -12.665  12.149  0.53 44.40
S
ATOM    312  CE  MET A  39      -2.908 -10.878  12.068  0.80 45.34
C
ATOM    313  N   CYS A  40      -3.760 -14.967  17.332  1.00 36.62
N
ATOM    314  CA  CYS A  40      -4.649 -15,-845  18.089  1.00 34.95
C
ATOM    315  C   CYS A  40      -5.444 -16.695  17.109  1.00 35.02
C
ATOM    316  O   CYS A  40      -6.008 -16.182  16.149  1.00 34.37
O
ATOM    317  CB  CYS A  40      -5.589 -15.032  18.996  1.00 33.32
C
ATOM    318  SG  CYS A  40      -7.025 -15.916  19.715  1.00 30.59
S
ATOM    319  N   PRO A  41      -5.493 -18.008  17.345  1.00 37.22
N
ATOM    320  CA  PRO A  41      -6.175 -18.878  16.376  1.00 36.05
C
ATOM    321  C   PRO A  41      -7.679 -18.622  16.323  1.00 35.38
C
ATOM    322  O   PRO A  41      -8.323 -19.057  15.381  1.00 35.44
O
ATOM    323  CB  PRO A  41      -5.925 -20.291  16.916  1.00 38.79
C
ATOM    324  CG  PRO A  41      -4.893 -20.158  17.999  1.00 40.0?
C
ATOM    325  CD  PRO A  41      -4.916 -18.745  18.484  1.00 35.53
C
ATOM    326  N   GLY A  42      -8.233 -17.970  17.341  1.00 34.46
N
ATOM    327  CA  GLY A  42      -9.671 -17.780  17.405  1.00 33.75
C
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ATOM    328  C   GLY A  42     --10.153 -16.408  16.950  1.00 34.93
C
ATOM    329  O   GLY A  42     --11.202 -16.299  16.333  1.00 33.81
0
ATOM    330  N   CYS A  43       -9.407 -15.357  17.275  1.00 33.06
N
ATOM    331  CA  CYS A  43       -9.853 -13.998  16.949  1.00 34.01
C
ATOM    332  C   CYS A  43       -8.862 -13.258  16.029,  1.00 34.94
C
ATOM    333  O   CYS A  43       -9.170 -12.186  15.494  1.00 35.97
O
ATOM    334  CB  CYS A  43     --10.095 -13.206  18.245  1.00 33.44
C
ATOM    335  SG  CYS A  43       -8.576 -12.552  18.929  1.00 30.93
S
ATOM    336  N   SER A  44       -7.680 -13.846  15.841  1.00 30.80
N
ATOM    337  CA  SER A  44       -6.623 -13.280  15.002  1.00 36.41
C
ATOM    338  C   SER A  44       -5.887 -12.045  15.554  1.00 37.05
C
ATOM    339  O   SER A  44       -5.013 -11.510  14.883  1.00 37.40
0
ATOM    340  CB  SER A  44       -7.127 -13.006  13.573  1.00 37.78
C
ATOM    341  OG  SER A  44       -7.544 -14.209  12.934  1.00 39.96
0
ATOM    342  N   ASP A  45       -6.203 -11.597  16.765  1.00 37.13
N
ATOM    343  CA  ASP A  45       -5.427 -10.504  17.345  1.00 37.68
C
ATOM    344  C   ASP A  45       -3.971 -10.937  17.469  1.00 41.55
C
ATOM    345  O   ASP A  45       -3.686 -12.129  17.584  1.00 41.64
0
ATOM    346  CB  ASP A  45       -5.961 -10.095  18.724  .1.00 37.82
C
ATOM    347  CG  ASP A  45       -7.058  -9.054  18.644  1.00 42.00
C
ATOM    348  OD1 ASP A  45       -7.441  -8.675  17.518  1.00 42,27
0
ATOM    349  OD2 ASP A  45       -7.553  -8.620  19.706  1.00 44.29
0
ATOM    350  N   PRO A  46       -3.037  -9.973  17.417  1.00 43.11
N
ATOM    351  CA  PRO A  46       -1.637 -10.254  17.748  1.00 43.08
C
ATOM    352  C   PRO A  46       -1.517 -10.858  19.140  1.00 38.17
C
ATOM    353  O   PRO A  46       -2.247 -10.466  20.039  1.0.0 39.81
O
ATOM    354  CB  PRO A  46       -0.995  -8.862  17.727  1.00 43.77
C
ATOM    355  CG- PRO A  46       -1.796  -8.119  16.696  1.00 44.17
C
ATOM    356  CD  PRO A  46       -3.216  -8.610  16.883  1.00 43.74
C
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ATOM 357 N VAL A 47 --0.607 -11.809 19.308 1.00 41.56
N
ATOM 35-8 CA VAL A 47 --0.352 -12.400 20.614 1.00 40.19
C
ATOM 359 C VAL A 47 1.050 -12.033 21.091 1.00 40.92
C
ATOM 360 0 VAL A 47 2.033 -12.319 20.409 1.00 43.59
0
ATOM 361 CB VAL A 47 --0.512 -13.929 20.574 1.00 40.22
C
ATOM 362 CGI VAL A 47 --0.285 -14.528 21.958 1.00 39.30
C
ATOM 363 CG2 VAL A 47 --1.903 -14.292 20.070 1.00 39.64
C
ATOM 364 N GLN A 48 1.122 -11.378 22.247 1.00 37.96
N
ATOM 365 CA GLN A 48 2.381 -10.977 22.867 1.00 39.29
C
ATOM 366 C GLN A 48 3.055 -12.186 23.504 1.00 42.53
C
ATOM 367 0 GLN A 48 4.248 -12.396 23.333 1.00 44.80
0
ATOM 368 CB GLN A 48 2.147 -9.900 23.939 1.00 45.07
C
ATOM 369 CG GLN A 48 1.609 -8.561 23.419 1.00 48.20
C
ATOM 370 CD GLN A 48 1.103 -7.642 24.534 1.00 50.16
C
ATOM 371 OE1 GLN A 48 1.450 -7.810 25.708 1.00 56.66
0
ATOM 372 NE2 GLN A 48 0.282 -6.660 24.165 1.00 52.21
N
ATOM 373 N ARG A 49 2.286 -12.978 24.247 1.00 41.50
N
ATOM 374 CA ARG A 49 2.821 -14.182 24.879 1.00 43.00
C
ATOM 375 C ARG A 49 1.706 -15.163 25.231 1.00 39.26
C
ATOM 376 O ARG A 49 0.530 -14.810 25.227 1.00 36.89
0
ATOM 377 CB ARG A 49 3.637 -13.834 26.135 1.00 42.27
C
ATOM 378 CG ARG A 49 2.814 -13.280 27.287 1.00 42.54
C
ATOM 379 CD ARG A 49 3.687 -12.917 28.491 1.00 44.32
C
ATOM 380 -NE ARG A 49 2.860 -12.525 29.636 1.00 46.91
N
ATOM 381 CZ ARG A 49 2.324 -11.319 29.779 1.00 48.55
C
ATOM 382 NH1 ARG A 49 2.531 -10.389 28.854 1.00 53.07
N
ATOM 383 NH2 ARG A 49 1.579 -11.041 30.836 1.00 50.50
N
ATOM 384 N ILE A 50 2.085 -16.399 25.526 1.00 40.07
N
ATOM 385 CA ILE A 50 1.111 -17.414 25.919 1.00 39.68
C

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ATOM    386  C   ILE A  50       1.328  -17.833  27.367  1.00  40.24
C
ATOM    387  0   ILE A  50       2.439  -18.207  27.733  1.00  45.42
0
ATOM    388  CB  ILE A  50       1.204  -18.648  25.014  1.00  39.47
C
ATOM    389 'CG1 ILE A  50       1.021  -18.247  23.548  1.00  40.35
C
ATOM    390  CG2 ILE A  50       0.159  -19.680  25.419  1.00  37.06
C
ATOM    391  CD1 ILE A  50       1.412  -19.333  22.574  1.00  40.23
C
ATOM    392  N   GLU A  51       0.276  -17.753  28.182  1.00  35.65
N
ATOM    393  CA  GLU A  51       0.320  -18.170  29.582  1.00  40.35
C
ATOM    394  C   GLU A  51      --0.148  -19.604  29.745  1.00  40.32
C
ATOM    395  O   GLU A  51      --1.108  -20.025  29.107  1.00  34.76
0
ATOM    396  CB  GLU A  51      --0.607  -17.311  30.433  1.00  41.50
C
ATOM    397  CG  GLU A  51      --0.241  -15.858  30.549  1.00  46.13
C
ATOM    398  CD  GLU A  51      --1.098  -15.176  31.590  1.00  46.41
C
ATOM    399  OE1 GLU A  51      --2.132  -15.767  31.994  1.00  44.73
0
ATOM    400  OE2 GLU A  51      --0.754  -14.050  31.989  1.00  49.07
0
ATOM    401  N   GLN A  52       0.514  -20.353  30.619  1.00  38.05
N
ATOM    402  CA  GLN A  52       0.064  -21.703  30.910  1.00  36.62
C
ATOM    403  C   GLN A  52      --0.926  -21.669  32.076  1.00 .39.43
C
ATOM    404  O   GLN A  52      --0.708  -20.979  33.087  1.00  40.34
0
ATOM    405  CB  GLN A  52       1.263  -22.626  31.180  1.00  41.16
C
ATOM    406  CG  GLN A  52       0.879  -24.071  31.374  1.00  42.97
C
ATOM    407  CD  GLN A  52       2.073  -24.942  31.746  1.00  47.68
C
ATOM    408  OE1 GLN A  52       3.167  -24.783  31.201  1.00  46.85
0
ATOM    409  NE2 GLN A  52       1.864  -25.861  32.683  1.00  50.11
N
ATOM    410  N   CYS A  53      --2.032  -22.390  31.926  1.00  34.22
N
ATOM    411  CA  CYS A  53      --3.144  -22.295  32.859  1.00  35.35
C
ATOM    412  C   CYS A  53      --3.805  -23.651  33.018  1.00  38.22
C
ATOM    413  O   CYS A  53      --4.101  -24.328  32.027  1.00  36.28
0
ATOM    414  CB  CYS A  53      --4.173  -21.294  32.325  1.00  38.17
C
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ATOM    415  SG  CYS A  53      -3.497  -19.649   32.029  1.00  46.52
S
ATOM    416  N   THR A  54      -4.022  -24.066   34.260  1.00  39.16
N
ATOM    417  CA  THR A  54      -4.683  -25.338   34.504  1.00  41.46
C
ATOM    418  C   THR A  54      -6.176  -25.129   34.378  1.00  43.34
C
ATOM    419  0   THR A  54      -6.672  -24.015   34.567  1.00  42.65
0
ATOM    420  CB  THR A  54      -4.359  -25.908   35.887  1.00  41.22
C
ATOM    421  OG1 THR A  54      -4.948  -25.068   36.886  1.00  46.40
0
ATOM    422  CG2 THR A  54      -2.847  -25.961   36.092  1.00  40.13
C
ATOM    423  N.  ARG A  55      -6.882  -26.211   34.065  1.00  46.70
N
ATOM    424  CA  ARG A  55      -8.299  -26.153   33.721  1.00  50.15
C
ATOM    425  C   ARG A  55      -9.124  -25.313   34.690  1.00  48.88
C
ATOM    426  0   ARG A  55      -9.914  -24.464   34.269  1.00  52.41
O
ATOM    427  CB  ARG A  55      -8.881  -27.569   33.650  1.00  49.70
C
ATOM    428  CG  ARG A  55      -7.838  -28.640   33.435  1.00  50.03
C
ATOM    429  CD  ARG A  55      -7.991  -29.788   34.421  1.00  53.70
C
ATOM    430  NE  ARG A  55      -9.257  -30.497   34.252  1.00  57.51
N
ATOM    431  CZ  ARG A  55      -9.372  -31.720   33.747  1.00  55.27
C
ATOM    432  NH1 ARG A  55      -8.294  -32.386   33.356  1.00  53.16
N
ATOM    433  NH2 ARG A  55     --10.571  -32.282   33.641  1.00  56.78
N
ATOM    434  N   GLY A  56      -8.945  -25.545   35.985  1.00  47.20
N
ATOM    435  CA  GLY A  56      -9.822  -24.943   36.980  1.00  45.88
C
ATOM    436  C   GLY A  56      -9.436  -23.565   37.486  1.00 -42.61
C
ATOM    437  O   GLY A  56     --10.011  -23.067   38.450  1.00  46.54
0
ATOM    438  N   SER A  57      -8.470  -22.932   36.842  1.00  39.66
N
ATOM    439  CA  SER A  57      -8.030  -21.622   37.290  1.00  39.65
C
ATOM    440  C   SER A  57      -8.649  -20.458   36.511  1.00  39.69
C
ATOM    441  O   SER A  57      -8.324  -19.302   36.777  1.00  36.93
0
ATOM    442  CB  SER A  57      -6.512  -21.521   37.201  1.00  40.98
C
ATOM    443  OG  SER A  57      -6.091  -21.673   35.863  1.00  43.18
O
```

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ATOM    444  N   LEU A  58      -9.537  -20.749   35.566  1.00 36.57
N
ATOM    445  CA  LEU A  58     --10.024  -19. 690  34 .666  1.00 36.78
C
ATOM    446  C   LEU A  58     --11.515  -19.368   34 .813  1.00 36.43
C
ATOM    447  0   LEU A  58     --12 .337  -20.241   35.136  1.00 33.28
0
ATOM    448  CB  LEU A  58      -9. 660  -20.024   33.213  1.00 34.54
C
ATOM    449  CG  LEU A  58      -8 .140  -19. 981  32 .965  1.00 38.83
C
ATOM    450  CD1 LEU A  58      -7.742  -20.499   31. 615  1.00 36.89
C
ATOM    451  CD2 LEU A  58      -7.583  -18.580   33. 178  1.00 38.56
C
ATOM    452  N   PHE A  59     --11.851  -18.099   34 .588  1.00 30.19
N
ATOM    453  CA  PHE A  59     --13.230  -17 .650  34 .635  1.00 27.99
C
ATOM    454  C   PHE A  59     --13.549  -17 .087  33.240  1.00 33.11
C
ATOM    455  0   PHE A  59     --13.038  -16.041   32.883  l'.OO 31.87
0
ATOM    456  CB  PHE A  59     --13.396  -16.555   35.670  1.00 32.16
C
ATOM    457  CG  PHE A  59     --13. 142  -17 .018  37 .082  1.00 32.53
C
ATOM    458  CD1 PHE A  59     --14 .185  -17.456   37. 875  1.00 34.55
C
ATOM    459  CD2 PHE. A  59     --11.855  -17 .018  37 .604  1.00 35.46
C
ATOM    460  CE1 PHE A  59     --13.959  -17.881   39.208  1.00 38.65
C
ATOM    461- CE2 PHE A  59     --11. 614  -17.440   38 .930  1.00 33.93
C
ATOM    462  CZ  PHE A  5      --12 .667  -17 .873  39.719  1.00 36.69
C
ATOM    463  N   MET A  60     --14 .378  -17 .792  32. 476  1.00 28.24
N
ATOM    464  CA  MET A  60     --14.619  -17 .445  31.066  1.00 28.77
C
ATOM    465  C   MET A  60     --15.888  -16.594   30.926  1.00 29.09
C
ATOM    466  O   MET A  60     --16. 909  -16.850   31.569  1.00 27.92
0
ATOM    467  CB  MET A  60     --14 .725 .-18.717   30. 199  1.00 30.69
C
ATOM    468  CG  MET A  60     --15. 102  -18 .458  28 .681  1.00 27.68
C
ATOM    469  SD  MET A  60     --15. 153  -19. 920  27 .622  1.00 24.08
S
ATOM    470  CE  MET A  60     --13. 406 -20.162   27.342  1.00 33.15
C
ATOM    471 ' N   CYS A  61     --15.816  -15.570   30.086  1.00 30.13
N
ATOM    472  CA  CYS A  61     --16. 999  -14.778   29.780  1.00 29-30
C
```

ATOM	473	C	CYS	A	61	−17.812	-15.524	28.717	1.00	26.48
C										
ATOM	474	0	CYS	A	61	−17.276	-15.876	27.. 6-87	1.00	27.76
0										
ATOM	475	CB	CYS	A	61	−16. 584	-13. 392	29.249	1.00	28.24
C										
ATOM	476	SG	CYS	A	61	−18.035	-12 .420	28.853	1.00	28.26
S										
ATOM	477	N	SER	A	62	−19. 085	-15.791	28. 975	1.00	26.86
N										
ATOM	478	CA	SER	A	62	−19. 893	-16. 494	27.982	1.00	31.16
C										
ATOM	479	C	SER	A	62	−20. 993	-15. 628	27.378	1.00	29.12
C										
ATOM	480	O	SER	A	62	−21. 983	-16.141	26.888	1.00	30.07
O										
ATOM	481	CB	SER	A	62	−20.514	-17.756	28.575	1.00	31.22
C										
ATOM	482	OG	SER	A	62	−21.347	-17.439	29. 678	1.00	36.37
O										
ATOM	483	N	ILE	A	63	−20.838	-14 .320	27.456	1.00	29.37
N										
ATOM	484	CA	ILE	A	63	−21.866	-13.420	26. 949	1.00	27.31
C										
ATOM	485	C	ILE	A	63	−21.860	-13.533	25.425	1.00	25.97
C										
ATOM	486	0	ILE	A	63	−20.803	-13.540	24.817	1.00	26.26
0										
ATOM	487	CB	ILE	A	63	−21. 601	-11. 990	27.394	1.00	29.81
C										
ATOM	488	CGI	ILE	A	63	−21. 991	-11.831	28.879	1.00	29.87
C										
ATOM	489	CG2	ILE	A	63	−22.374	-10.983.	26.516	1.00	28.87
C										
ATOM	490	CD1	ILE	A	63	−21.453	-10. 589	29. 496	1.00	33.99
C										
ATOM	491	N	VAL	A	64	−23.053	-13. 646	24.846	1.00	25.62
N										
ATOM	492	CA	VAL	A	64	−23.255	-13.,779	23. 401	1.00	27.13
C										
ATOM	493	C	VAL	A	64	−23. 928	-12 .489	22.898	1.00	27.28
C										
ATOM	494	O	VAL	A	64	−25.052	-12.185	23.294	1.00	26.51
0										
ATOM	495	CB	VAL	A	64	−24.182	-14 .963	23.088	1.00	25.78
C										
ATOM	496	CGI	VAL	A	64	−24.352	-15. 150	21.576	1.00	25. 68
C										
ATOM	497	CG2	VAL	A	64	−23.647	-16.264	23.747	1.00	32.01
C										
ATOM	498	N	GLN	A	65	−23.214	-11.717	22.082	1.00	28.06
N										
ATOM	499	CA	GLN	A	65	−23.769	-10.487	21.517	1.00	28.01
C										
ATOM	500	C	GLN	A	65	−23.390	-10.383	20.050	1.00	28.25
C										
ATOM	501	O	GLN	A	65	−22.483	-11.072	19.588	1.00	26.46
0										

ATOM 502 CB GLN A 65 −23.247 -9.266 22.265 1.00 31.43
C
ATOM 503 CG GLN A 65 −23. 887 -9.035 23. 613 1.00 34.36
C
ATOM 504 CD- GLN A 65 −23. 152 -8 .008 24 .430 1.00 33.33
C
ATOM 505 OE1 GLN A 65 −21. 928 -7.885 24.337 1.00 32.76
0
ATOM 506 NE2 GLN A 65 −23.896 -7.260 25.253 1.00 34.07
N
ATOM 507 N GLY A 66 −24 .059 -9. 494 19. 322 1.00 29.44
N
ATOM 508 CA GLY A 66 −23. 681 -9.230 17. 945 1.00 26.86
C
ATOM 509 C GLY A 66 −22.366 -8 .463 17.836 1.00 25.43
C
ATOM 510 O GLY A 66 −21. 961 -7.740 18.750 1.00 28.16
0
ATOM 511 N CYS A 67 −21. 692 -8 .625 16.702 1.00 27.03
N
ATOM 512 CA CYS A 67 −20.429 -7 .948 16.426 1.00 26.02
C
ATOM 513 C CYS A 67 −20. 612 -6.441 16.213 1.00 28.23
C
ATOM 514 O CYS A 67 −20. 018 -5. 631 16. 912 1 .00 25 .82
0
ATOM 515 CB CYS A 67 −19.794 -8 .549 15.171 1.00 30.57
C
ATOM 516 SG CYS A 67 −18.247 -7 .773 14 .685 1.00 29.97
S
ATOM 517. N LYS A 68 −21.413 -6. 095 15.206 1.00 27.96.
N
ATOM 518 CA LYS A 68 −21.794 -4 .705 14 .901 1.00 28.07
C
ATOM 519 C LYS A 68 −20. 683 -3.786 14 .394 1.00 28.78
C
ATOM 520 0 LYS A 68 −20. 910 -2.584 14 .293 1.00 30.23
0
ATOM 521 CB LYS A 68 −22.509 -4 .053 16.088 1.00 27.80
C
ATOM 522 CG LYS A 68 −23. 660 -4 .893 16. 623 1.00 31.00
C
ATOM 523 CD LYS A 68 −24 .513 -4 .103 17: 586 1.00 32.54
C
ATOM 524 CE LYS A 68 −25.516. -5.006 18.309 1.00 36.47
C
ATOM 525 NZ LYS A 68 −26. 482 -4 .203 19. 117 1.00 37.71
N
ATOM 526 N ARG A 69 −19. 498 -4 .322 14 .096 1.00 27.32
N
ATOM 527 CA ARG A 69 −18.454 -3. 527 13.445 1.00 26.84
C
ATOM 528 C ARG A 69 −18 .908 -3.233 12 .020 1.00 25.54
C .
ATOM 529 O ARG A 69 −19. 608 -4 .041 11.432 1.00 24.67
0
ATOM 530 CB ARG A 69 −17 .118 -4.275 13. 396 1.00 27.49
C

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ATOM    531  CG  ARG A  69     -16.516  -4.546  14.794  1.00 27.95
C
ATOM    532  CD  ARG A  69     -15.050  -5.003  14.645  1.00 31.81
C
ATOM    533  NE  ARG A  69     -14.586  -5.745  15.821  1.00 34.85
N
ATOM    534  CZ  ARG A  69     -14.100  -5.190  16.930  1.00 37.73
C
ATOM    535  NH1 ARG A  69     -14.013  -3.873  17.042  1.00 33.68
N
ATOM    536  NH2 ARG A  69     -13.699  -5.959  17.941  1.00 37.33
N
ATOM    537  N   THR A  70     -18.493  -2.082  11.488  1.00 25.54
N
ATOM    538  CA  THR A  70     -18.948  -1.615  10.169  1.00 24.97
C
ATOM    539  C   THR A  70     -17.802  -1.500   9.193  1.00 27.68
C
ATOM    540  O   THR A  70     -16.672  -1.191   9.587  1.00 27.19
0
ATOM    541  CB  THR A  70     -19.660  -0.236  10.208  1.00 24.25
C
ATOM    542  OG1 THR A  70     -18.789   0.765  10.747  1.00 28.64
O
ATOM    543  CG2 THR A  70     -20.927  -0.305  11.060  1.00 27.10
C
ATOM    544  N   TYR A  71     -18.126  -1.688   7.909  1.00 27.34
N
ATOM    545  CA  TYR A  71     -17.134  -1.679   6.855  1.00 28.22
C
ATOM    546  C   TYR A  71     -17.659  -0.806   5.717  1.00 31.88
C
ATOM    547  O   TYR A  71     -18.871  -0.592   5.623  1.00 28.57
0
ATOM    548  CB  TYR A  71     -16.785  -3.113   6.420  1.00 30.27
C
ATOM    549  CG  TYR A  71     -16.130  -3.779   7.607  1.00 29.96
C
ATOM    550  CD1 TYR A  71     -14.777  -3.626   7.840  1.00 30.86
C
ATOM    551  CD2 TYR A  71     -16.897  -4.427   8.558  1.00 31.39
C
ATOM    552  CE1 TYR A  71     -14.176  -4.165   8.977  1.00 33.06
C
ATOM    553  CE2 TYR A  71     -16.305  -4.980   9.719  1.00 31.59
C
ATOM    554  CZ  TYR A  71     -14.949  -4.844   9.901  1.00 30.89
C
ATOM    555  OH  TYR A  71     -14.343  -5.342  11.032  1.00 33.17
0
ATOM    556  N   LEU A  72     -16.744  -0.294   4.897  1.00 32.47
N
ATOM    557  CA  LEU A  72     -17.106   0.651   3.828  1.00 34.44
C
ATOM    558  C   LEU A  72     -17.426  -0.059   2.520  1.00 34.37
C
ATOM    559  O   LEU A  72     -17.977   0.539   1.583  1.00 32.44
0
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ATOM 560 CB LEU A 72 --16.008 1.707 3.648 1.00 33.04
C
ATOM 561 CG LEU A 72 --15.725 2.532 4.910 1.00 37.77
C
ATOM 562 CD1 LEU A 72 --14.622 3.565 4.700 1.00 43.02
C
ATOM 563 CD2 LEU A 72 --16.996 3.190 5.425 1.00 37.13
C
ATOM 564 N SER A 73 --17.112 -1.351 2.460 1.00 31.48
N
ATOM 565 CA SER A 73 --17.438 -2.152 1.297 1.00 34.16
C
ATOM 566 C SER A 73 --17.814 -3.555 1.727 1.00 36.21
C
ATOM 567 O SER A 73 --17.384 -4.035 2.798 1.00 32.85
0
ATOM 568 CB SER A 73 --16.244 -2.220 0.340 1.00 35.70
C
ATOM 569 OG SER A 73 --15.226 -3.083 0.845 1.00 35.50
0
ATOM 570 N GLN A 74 --18.605 -4.217 0.889 1.00 32.46
N
ATOM 571 CA GLN A 74 --19.006 -5.579 1.170 1.00 37.49
C
ATOM 572 C GLN A 74 --17.761 -6.452 1.187 1.00 39.18
C
ATOM 573 O GLN A 74 --17.623 -7.344 2.028 1.00 35.24
0
ATOM 574 CB GLN A 74 --20.018 -6.066 0.144 1.00 38.66
C
ATOM 575 CG GLN A 74 --20.398 -7.513 0.315 1.00 39.76
C
ATOM 576 CD GLN A 74 --21.084 -7.770 1.628 1.00 44.01
C
ATOM 577 OE1 GLN A 74 --21.715 -6.873 2.202 1.00 44.47
0
ATOM 578 NE2 GLN A 74 --20.966 -9.002 2.123 1.00 43.00
N
ATOM 579 N ARG A 75 --16.840 -6.175 0.269 1.00 35.46
N
ATOM 580 CA ARG A 75 --15.565 -6.879 0.236 1.00 37.99
C
ATOM 581 C ARG A 75 --14.797 -6,793 1.567 1.00 36.30
C
ATOM 582 O ARG A 75 --14.220 -7.793 2.037 1.00 35.88
0
ATOM 583 CB ARG A 75 --14.702 -6.345 -0.914 1.00 42.31
C
ATOM 584 CG ARG A 75 --13.287 -6.886 -0.939 1.00 45.80
C
ATOM 585 CD ARG A 75 --12.512 -6.368 -2.155 1.00 47.25
C
ATOM 586 NE ARG A 75 --13.005 -6.943 -3.404 1.00 50.42
N
ATOM 587 CZ ARG A 75 −12.604 -6.555 -4.614 1.00 50.59
C
ATOM 588 NH1 ARG A 75 --11.711 -5.582 -4.746 1.00 50.87
N

ATOM 589 NH2 ARG A 75 −13.102 -7.132 -5.68-9 1.00 49.38
N
ATOM 590 N ASP A 76 −14.762 -5.605 2.164 1.00 33.72
N
ATOM 591 CA ASP A 76 −14.075 -5.439 3.444 1.00 33.53
C
ATOM 592 C ASP A 76 −14.810 -6.177 4.566 1.00 33.79
C
ATOM 593 O ASP A 7_6 −14.180 -6.750 5.464 1.00 33.84
O
ATOM 594 CB ASP A 76 −13.907- -3.961 3.796 1.00 34.76
C
ATOM 595 CG ASP A 76 −12.816 -3.281 2.968 1.00 37.59
C
ATOM 596 OD1 ASP A 76 −12.127 -3.980 2.190 1.00 37.52
0
ATOM 597 OD2 ASP A 76 −12.656 -2.050 3.092 1.00 34.86
0
ATOM 598 N LEU A 77 −16.136 -6.173 4.516 1.00 32.61
N
ATOM 599 CA LEU A 77 −16.914 -6.920 5.507.- 1.00 32.90
C
ATOM 600 C LEU A 77 −16.631 -8.411 5.394 1.00 35.63
C
ATOM 601 O LEU A 77 −16.482 -9.100 6.411 1.00 29.68
0
ATOM 602 CB LEU A 77 −18.406 -6.662 5.343 1.00 33.65
C
ATOM 603 CG LEU A 77 −19.378 -7.549 6.123 1.00 33.62
C
ATOM 604 CD1 LEU A 77 −19.113 -7,475 7.643 1.00 32.25
C
ATOM 605 CD2 LEU A 77 −20.803 -7.133 5.798 1.00 33.57
C
ATOM 606 N GLN A 78 −16.554 -8.915 4.159 1.00 33.82
N
ATOM 607 CA GLN A 78 −16.246 -10.319 3.962 1.00 34.23
C
ATOM 608 C GLN A 78 −14.838 -10.655 4.464 1.00 36.22
C
ATOM 609 O GLN A 78 −14.630 -11.688 5.114 1.00 34.64
0
ATOM 610 CB GLN A 78 −16.435 -10.738 2.495 1.00 37.69
C
ATOM 611 CG GLN A 78 −16.346 -12.241 2.304 1.00-40.01
C
ATOM 612 CD GLN A 78 −17.376 -12.995 3.124 1.00 42.39
C
ATOM 613 OE1 GLN A 78 −18.573 -12.694 3.073 1.00 44.55
O
ATOM 614 NE2 GLN A 78 −16.917 -13.982 3.889 1.0D 43.06
N
ATOM 615 N ALA A 79 −13.875 -9.779 4.193 1.00 34.75
N
ATOM 616 CA ALA A 79 −12.520 -10.000 4.692 1.00 36.58
C
ATOM 617 C ALA A 79 −12.480 -10.019 6.228 1.00 35.62
C

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ATOM    618  0   ALA A  79      --11.756 -10.829   6.836  1.00 33.94
0
ATOM    619  CB  ALA A  7.9     --11.565  -8. 964  4.142  1.00 37.21
C
ATOM    620  N   HIS A  80      --13.262  -9.139   6.849  1.00 31.82
N
ATOM    621  CA  HIS A  80      --13.408  -9.126   8.309  1.00 32.56
C
ATOM    622  C   HIS A  80      --13.974 -10.441   8.859  1.00 32.70
C
ATOM    623  O   HIS A  80      --13.490 -10.966   9.870  1.00 33.75
0
ATOM    624  CB  HIS A  80      -14.286  -7.940   8.748  1.00 29.-70
C
ATOM    625  CG  HIS A  80      --14.909  -8.112  10.100  1.00 29.10
C
ATOM    626  ND1 HIS A  80      --14.187  -7.980  11.269  1.00 30.37
N
ATOM    627  CD2 HIS A  80      --16.176  -8.414  10.-472  1.00 28.07
C
ATOM    628  CE1 HIS A  80      --14.987  -8.172  12.302  1.00 29.59
C
ATOM    629  NE2 HIS A  80      --16.197  -8.442  11.850  1.00 31.18
N -
ATOM    630  N   ILE A  81      --15.012 -10.955   8.209  1.00 31.23
N
ATOM    631  CA  ILE A  81      --15.630 -12.215   8.598  1.00 32.10
C
ATOM    632  C   ILE A  81      --14.624 -13.369   8.476  1.00 34.77
C
ATOM    633  0   ILE A  81      --14.532 -14.217   9.379  1.00 32.08
0
ATOM    634  CB  ILE A  81      --16.906 -12.513   7.756  1.00 35.04
C
ATOM    635  CGI ILE A  81      --17.989 -11.459   8.016  1.00 32.21
C
ATOM    636  CG2 ILE A  81      --17.446 -13.912   8.052  1.00 36.43
C
ATOM    637  CD1 ILE A  81      --19.226 -11.632   7.119  1.00 36.21
C
ATOM    638  N   ASN A  82      --13.871 -13.404   7.372  1.00 34.-36
NT
ATOM    639  CA  ASN A  82      --12.858 -14.441   7.176  1.00 36.28
C
ATOM    640  C   ASN A  82      --11.737 -14.366   8.207  1.00 38.04
C
ATOM    641  0   ASN A  82      --11.144 -15.390   8.573  1.00 38.73
0
ATOM    642  CB  ASN A  82      --12.255 -14.375   5.761  1.00 38. 99
C
ATOM    643  CG  ASN A  82      --13.242 -14.775   4 .684  1.00 41.72
C
ATOM    644  OD1 ASN A  82      --14.334 -15.265   4.976  1.00 42.85
0
ATOM    645  ND2 ASN A  82      --12.858 -14.575   3.420  1.00 45.66
N
ATOM    646  N   HIS A  83      --11.460 -13.153   8.675  1.00 35.37
N
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ATOM    647  CA  HIS A  83     -10.331  -12.875    9.554  1.00 34.29
C
ATOM    648  C   HIS A  83     -10.701  -13.089   11.020  1.00 35.78
C
ATOM    649  0   HIS A  83      -9.883  -13.538   11.818  1.00 35.91
0
ATOM    650  CB  HIS A  83      -9.873  -11.426    9.329  1.00 35.58
C
ATOM    651  CG  HIS A  83      -8.599  -11.059   10.028  1.00 39.32
C
ATOM    652  ND1 HIS A  83      -7.400  -11.692    9.779  1.00 41.42
N
ATOM    653  CD2 HIS A  83      -8.333  -10.101   10.949  1.00 38.45
C
ATOM    654  CE1 HIS A  83      -6.454  -11.149   10.525  1.00 39.51
C
ATOM    655  NE2 HIS A  83      -6.994  -10.181   11.243  1.00 39.21
N
ATOM    656  N   ARG A  84     -11.945  -12.778   11.364  1.00 32.69
N
ATOM    657  CA  ARG A  84     -12.347  -12.706   12.753  1.00 31.89
C
ATOM    658  C   ARG A  84     -13.289  -13.803   13.232  1.00 32.14
C
ATOM    659  O   ARG A  84     -13.349  -14.071   14.455  1.00 32.10
0
ATOM    660  CB  ARG A  84     -13.025  -11.353   13.033  1.00 34.34
C
ATOM    661  CG  ARG A  84     -12.160  -10.131   12.762  1.00 31.51
C
ATOM    662  CD  ARG A  84     -10.873  -10.141   13.567  1.00 36.82
C
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C
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N
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C
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C
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C
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N
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C
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C
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C
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O
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C
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ZN
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ZN
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ZN
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0
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HETATM  783  O   HOH A 128     -17.813  -19.489   12.355  1.00 44.34
0
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O
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O
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CONECT   57  758
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CONECT  172  759
CONECT  199  758
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CONECT  335  759
CONECT  516  757
CONECT  629  757
CONECT  676  757
CONECT  757  516  629  676
CONECT  758   34   57  199  225
CONECT  759  156  172  318  335
MASTER      326    0    3    3    3    0    3    6  828    1   14    8
END
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CLAIMS

1. A drug screening method comprising:

a)contacting an isolated polypeptide with a compound wherein the polypeptide comprises or consists of the following phosphotyrosine-binding domain:

TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto wherein the following amino acids are conserved C166, C172, H185, and H190;

b)determining whether binding occurs between the polypeptide and the compound; and

c)where said binding occurs concluding said compound may be useful in preventing the degradation of proteins that bind with said polypeptide.

2.The method according to claim 1 wherein the phosphotyrosine-binding domain is characterised by sequence structure:

TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH

(a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto.

3. The method according to claim 1 wherein the polypeptide is characterised by sequence structure:

VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIEQ CTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH

(a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23 % homologous thereto wherein the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190.

4. The method according to claim 1 wherein the polypeptide is characterised by sequence structure:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIEQ
CTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 106-206) SEQ ID NO: 2 or a sequence at least 71% homologous thereto.

5. The method according to claim 1 wherein said polypeptide comprises a phosphotyrosine-binding domain characterised by two of the following sequence structures:
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto where the following amino acids are conserved C166, C172, H185, and H190, arranged as a dimer.

6. The method according claim 1 wherein said polypeptide comprises a phosphotyrosine-binding domain characterised by two of the following sequence structures:
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto, arranged as a dimer.

7. The method according claim 1 wherein said polypeptide is characterised by two of the following sequence structures:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIEQ
CTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH
(a.a. 106-206) SEQ ID NO: 2 or a sequence at least 23% homologous thereto where the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190, arranged as a dimer.

8. The method according claim 1 wherein said polypeptide is characterised by two of the following sequence structures:

VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIEQ CTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH (a.a. 106-206) SEQ ID NO: 2 or a sequence at least 71% homologous thereto, arranged as a dimer.

9. The method according to any one of claims 5-8 wherein said dimer is an anti-parallel dimer.

10. The method according to any preceding claim wherein said polypeptide is a ubiquitin 3 ligase or a fragment thereof.

11. The method according to any preceding claim wherein said polypeptide is selected from the group comprising Hakai, ZNF645, Ligand-of-Numb protein X1 and Ligand-of-Numb protein X2, or a fragment thereof.

12. The method according to any preceding claim wherein said polypeptide binds E-cadherin or DOK1 or cortacin.

13. The method according to any preceding claim wherein said polypeptide protein has the following conserved target binding residues H127 and H185.

14. The method according to claim 14 wherein the polypeptide further has the following conserved target binding residues R189 and/or Y176.

15. The method according to any preceding claim wherein, under part c), where said binding occurs concluding said compound may be useful in preventing cell migration or metastasis or invasion or cancer.

16. The method according to any one of claims 1, 3, 5 or 7 wherein said sequence homology is 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44% or 45%.

17. The method according to any one of claims 2, 4, 6, or 8 wherein said sequence homology is 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

18. The method according to any preceding claim wherein said binding under part c) may be determined either *in vitro, in vivo* or *in silico.*

19. An isolated polypeptide selected from the group comprising:

i)TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto wherein the following amino acids are conserved C166, C172, H185, and H190;

ii)TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLE NVH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto;

iii)VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDP VQRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPV TRASLENVH SEQ ID NO: 2 (a.a. 106-206) or a sequence at least 23 % homologous thereto wherein the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 €148, C166, C172, H185, and H190;

iv)VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDP VQRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPV TRASLENVH SEQ ID NO: 2 (a.a. 106-206) or a sequence at least 71% homologous thereto;

v)two of the following sequence structures:

TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 31% homologous thereto where the following amino acids are conserved C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer;

vi)two of the following sequence structures:
TRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLEN
VH (a.a. 159-206) SEQ ID NO:1 or a sequence at least 76% homologous thereto arranged as a dimer, ideally an anti-parallel dimer;
vii)two of the following sequence structures:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPV
QRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVT
RASLENVH (a.a. 106-206) SEQ ID NO:2 or a sequence at least 23% homologous thereto where the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190, arranged as a dimer, ideally an anti-parallel dimer;
viii)two of the following sequence structures:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGCSDPV
QRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHMRAGKPVT
RASLENVH SEQ ID NO: 2 (a.a. 106-206) SEQ ID NO:2 or a sequence at least 71% homologous thereto arranged as a dimer, ideally an anti-parallel dimer;
ix)an isolated polypeptide according to i), ii), v) and vi) in combination with a RING domain characterized by sequence structure:
VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPGC (a.a. 106-148) SEQ ID NO:3;
x)IHFCDKCDLPIKIYGRIIPCKHAFCYHCANLYDKVGYKVCPRCRYPV
LRIEAHKRGSVFMCSIVQQCKRTYLSQKSLQAHIKRRHKRARKQVTS
ASLEKVR (a.a. 54-154 ZNF645) SEQ ID NO:4 or a sequence at least 71% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190;
xi) DLVCHICLLQPLLQPLDTPCGHTFCYKCLRNFLQEKDFCPLDRKRL
HFKLCKKSSILVHKLLDKLLVLCPFSSVCKDVMQRCDLEAHLKNRCP
GASHRRVALERRKTS (a.a. 47-153 LNX2) or a sequence at least

25% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 C148, C166, C172, H185, and H190; and

xii)DLICHICLQALLDPLDTPCGHTYCTLCLTNFLVEKDFCPMDRKPLV LQHCKKSSILVNKLLNKLLVTCPFREHCT-QVLQRCDLEHHFQTSCKGASHYGLTKDRKRRS (a.a. 38-144 LNX1) or a sequence at least 23% homologous thereto wherein, when aligned with Hakai a.a. 106-206, the following amino acids are conserved C109, C112, C125, H127, C130, C133, C145 €148, C166, C172, H185, and H190.

20. Use of Methotraxate Hydrate, or a derivative or salt thereof, to treat a disease characterised by migration or metastasis or invasion or a lack of cell-cell adhesion.

21. Use of Methotraxate Hydrate, or a derivative or salt thereof, in the manufacture of a medicament to treat a disease characterised by migration or metastasis or invasion or a lack of cell-cell adhesion.

22. The Use according to claim 20 or 21 wherein said disease is cancer, dysplasia or hyperplasia.

23. A crystal form of the isolated polypeptide according to claim 19 wherein said crystal is characterised by the co-ordinates and structure factors described in Table 3.

Figure 1

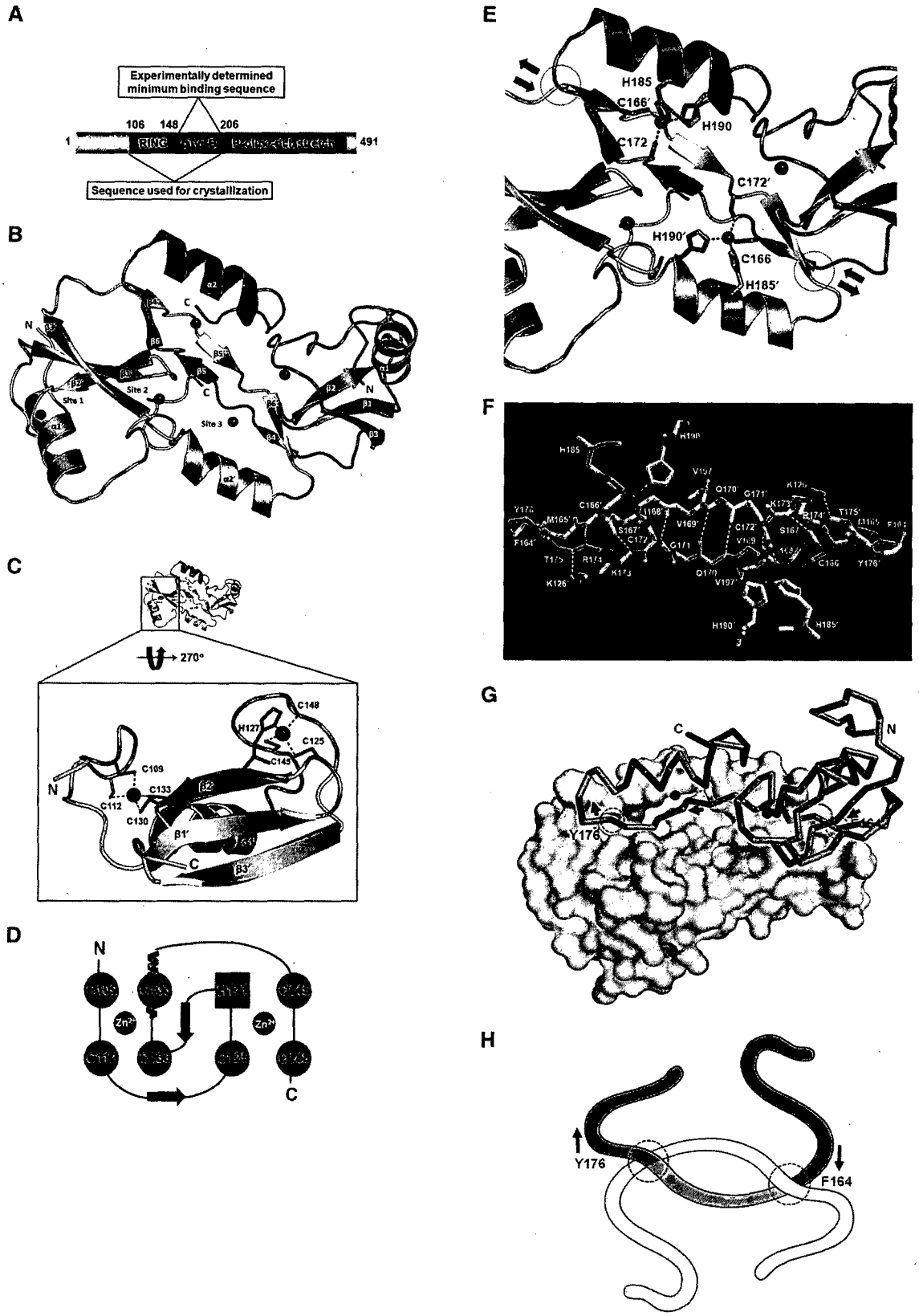

A
Experimentally determined minimum binding sequence
106
148
206
1
491
Sequence used for crystallization
B
N
C
Site 1
Site 2
Site 3
C
N
C
270°
C148
H127
C125
C109
C133
C112
C130
N
C
N
Zn2+
Zn2+
C
E
H185
C166'
H190
C172
C172'
H190'
C166
H185'
F
H185
H190
Q170'
G171'
C166'
V169'
S167'
C172'
G171
T175'
R174'
K173'
K126'
Q170'
V197'
C130
Y176
H190'
H185'
G
C
N
Y176
H
Y176
F164

Figure 2

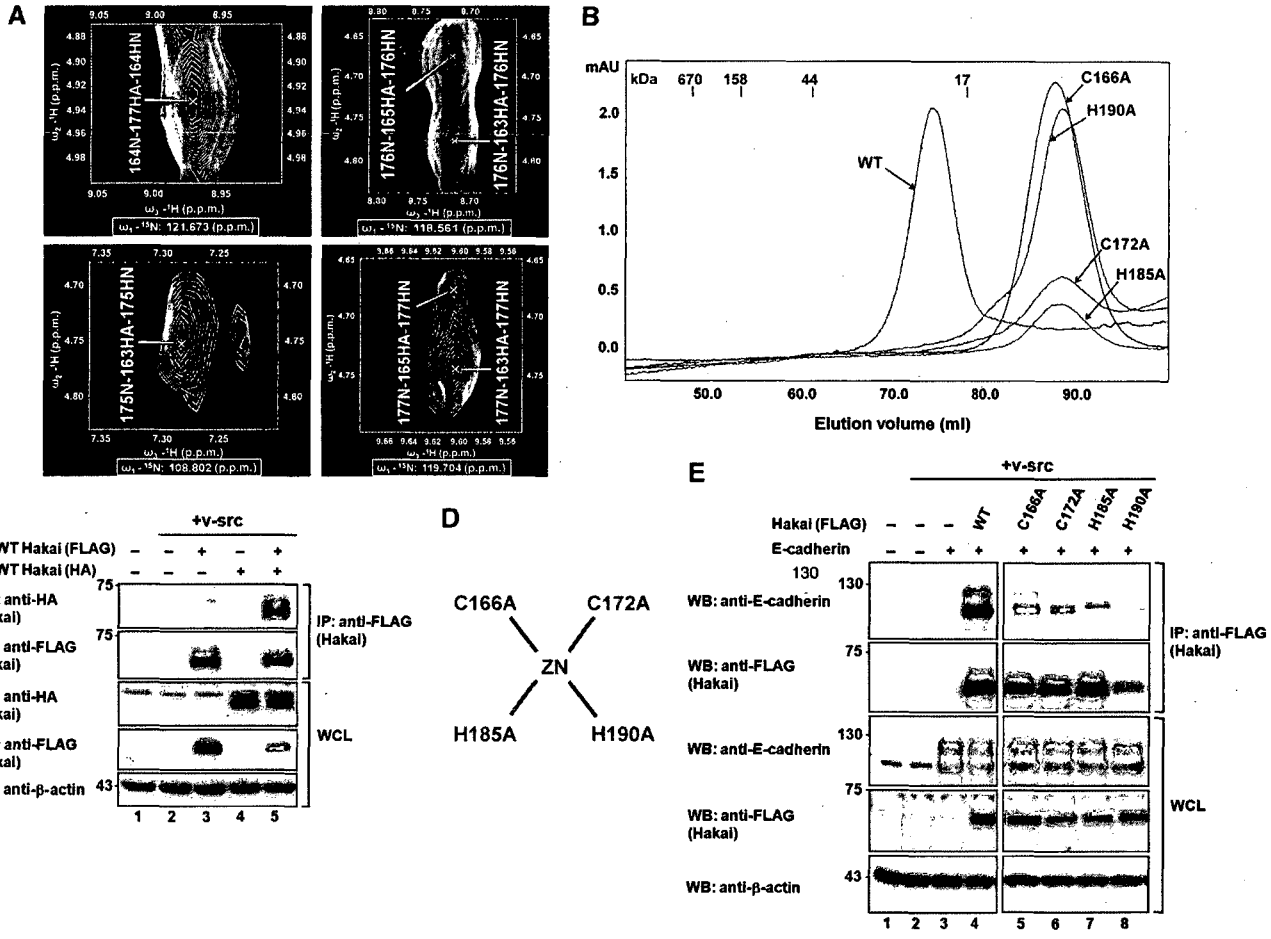
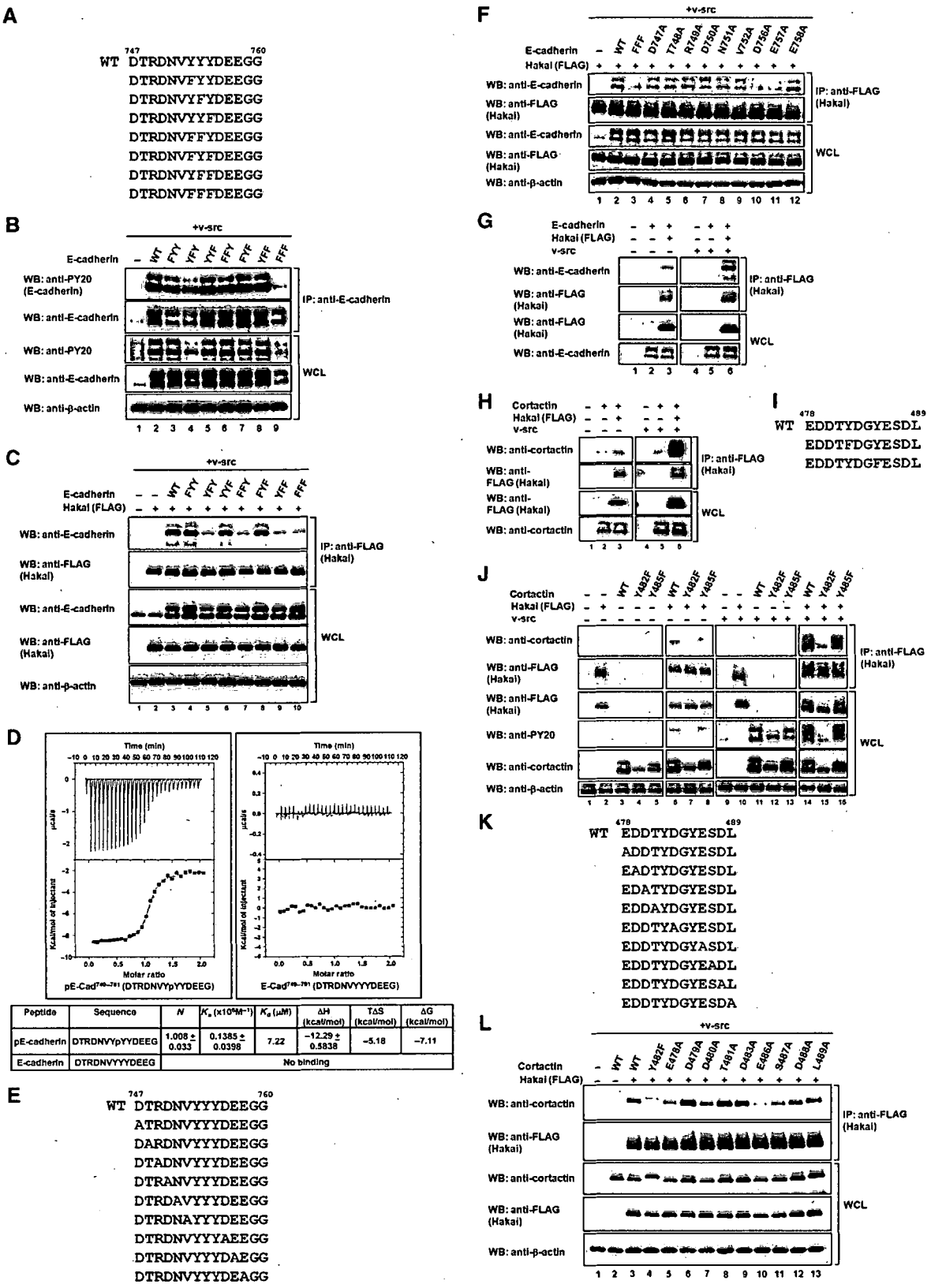

A
164N-177HA-164HN
176N-165HA-176HN
176N-163HA-176HN
175N-163HA-175HN
177N-165HA-177HN
177N-163HA-177HN
B
mAU
kDa
670
158
44
17
C166A
H190A
WT
C172A
H185A
Elution volume (ml)
C
+v-src
WT Hakai (FLAG)
WT Hakai (HA)
WB: anti-HA (Hakai)
WB: anti-FLAG (Hakai)
WB: anti-HA (Hakai)
WB: anti-FLAG (Hakai)
WB: anti-β-actin
IP: anti-FLAG (Hakai)
WCL
D
C166A
C172A
ZN
H185A
H190A
E
+v-src
Hakai (FLAG)
E-cadherin
WT
C166A
C172A
H185A
H190A
WB: anti-E-cadherin
WB: anti-FLAG (Hakai)
WB: anti-E-cadherin
WB: anti-FLAG (Hakai)
WB: anti-β-actin
IP: anti-FLAG (Hakai)
WCL

Figure 3



Peptide	Sequence	N	K_a ($x10^6 M^{-1}$)	K_a (μM)	ΔH (kcal/mol)	TΔS (kcal/mol)	ΔG (kcal/mol)
pE-cadherin	DTRDNVYpYYDEEG	1.008 ± 0.033	0.1385 ± 0.0398	7.22	−12.29 ± 0.5838	−5.18	−7.11
E-cadherin	DTRDNVYYYDEEG	No binding					

Figure 4

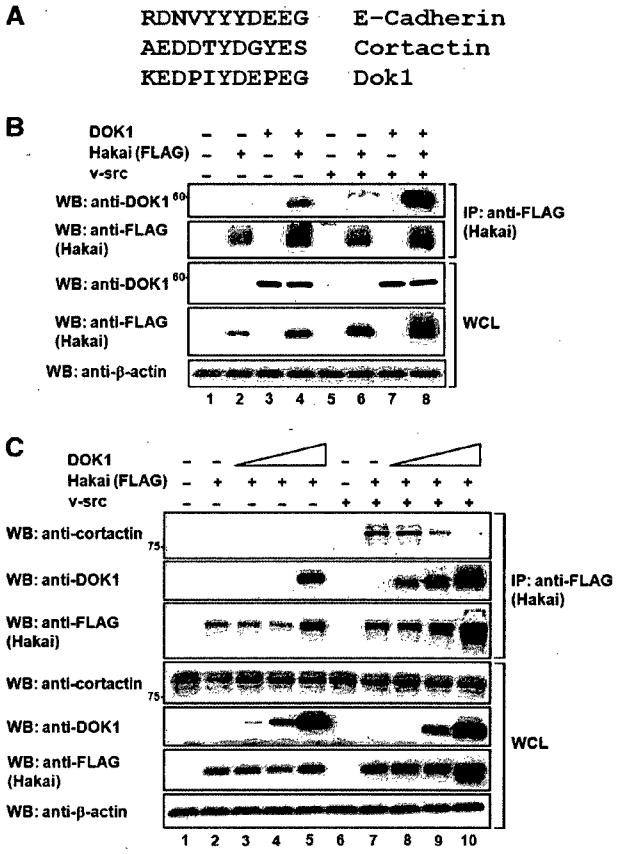

A
RDNVYYYDEEG E-Cadherin
AEDDTYDGYES Cortactin
KEDPIYDEPEG Dok1
B
DOK1
Hakai (FLAG)
v-src
WB: anti-DOK1
WB: anti-FLAG (Hakai)
WB: anti-DOK1
WB: anti-FLAG (Hakai)
WB: anti-β-actin
IP: anti-FLAG (Hakai)
WCL
1 2 3 4 5 6 7 8
C
DOK1
Hakai (FLAG)
v-src
WB: anti-cortactin
WB: anti-DOK1
WB: anti-FLAG (Hakai)
WB: anti-cortactin
WB: anti-DOK1
WB: anti-FLAG (Hakai)
WB: anti-β-actin
IP: anti-FLAG (Hakai)
WCL
1 2 3 4 5 6 7 8 9 10

Figure 5

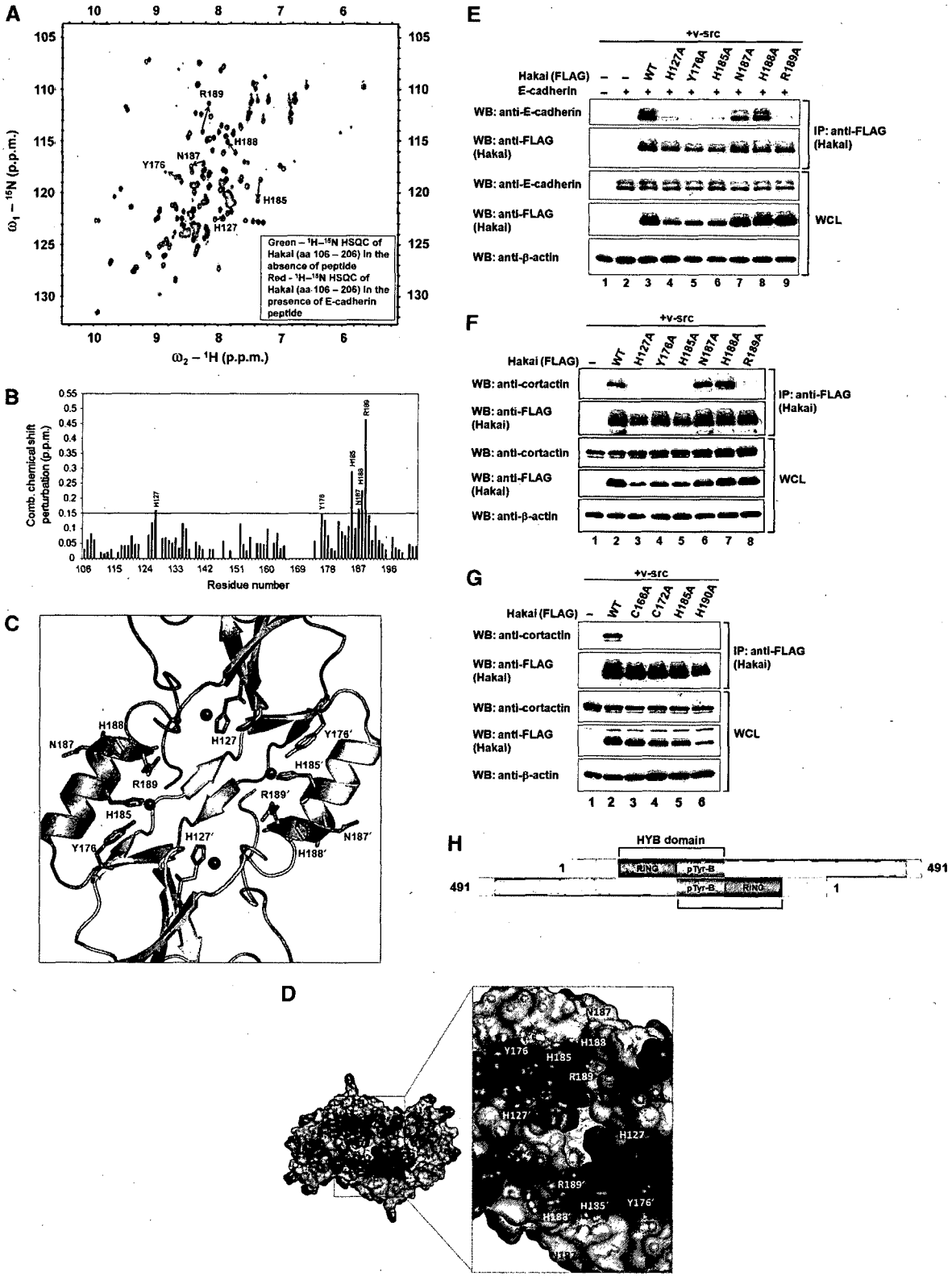
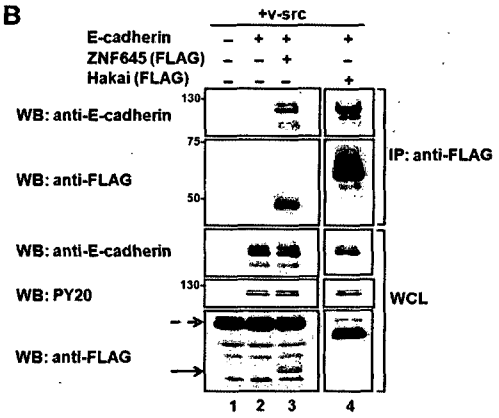
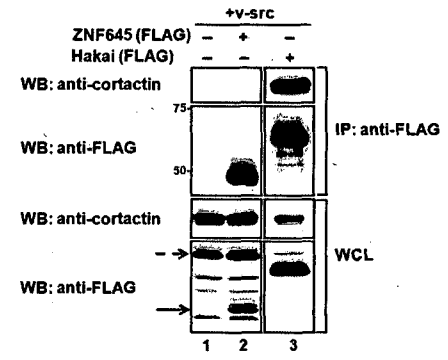

A
B
C
D
E
F
G
H
Green – ¹H–¹⁵N HSQC of Hakai (aa 106 – 206) in the absence of peptide
Red - ¹H–¹⁵N HSQC of Hakai (aa 106 – 206) in the presence of E-cadherin peptide
Residue number
Comb. chemical shift perturbation (p.p.m.)
+v-src
Hakai (FLAG)
E-cadherin
WT
H127A
Y176A
H185A
N187A
H188A
R189A
WB: anti-E-cadherin
WB: anti-FLAG (Hakai)
WB: anti-β-actin
IP: anti-FLAG (Hakai)
WCL
WB: anti-cortactin
C166A
C172A
H190A
HYB domain
RING
pTyr-B

Figure 6

A

```
Hakai    127 HVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRTYLSQRDLQAHINHRHM 191
ZNF645    75 HAFCYHCANLYDKVGYKVCPRCRYPVLRIEAHKRGSVFMCSIVQQCKRTYLSQKSLQAHIKRRHK 139
```





D

```
Human_Hakai    106 VHFCDKCGLPIKIYGRMIPCKHVFCYDCAILHEKKGDKMCPG---------CSDPVQRIEQCTRGSLFMC 166
Human_Znf645    54 IHFCDKCDLPIKIYGRIIPCKHAFCYHCANLYDKVGYKVCPR---------CRYPVLRIEAHKRGSVFMC 114
Human_Lnx2      47 DLVCHICLQPLLQP-LDTPCGHTFCYKCLRNFLQEKD-FCPLDRKRLHFKLCKKSSILVHKLLDKLLVLC 114
Human_Lnx1      38 DLICHICLQALLDP-LDTPCGHTYCTLCLTNFLVEKD-FCPMDRKPLVLQHCKKSSILVNKLLNKLLVTC 105

Human_Hakai    167 SIVQGCKRTYLSQRDLQAHINHRHMRAGKPVTRASLENVH 206
Human_Znf645   115 SIVQQCKRTYLSQKSLQAHIKRRHKRARKQVTSASLEKVR 154
Human_Lnx2     115 PFSSVCK-DVMQRCDLEAHLKNRCPGASHRRVALERRKTS 153
Human_Lnx1     106 PFREHCT-QVLQRCDLEHHFQTSCKGASHYGLTKDRKRRS 144
```

Figure 7

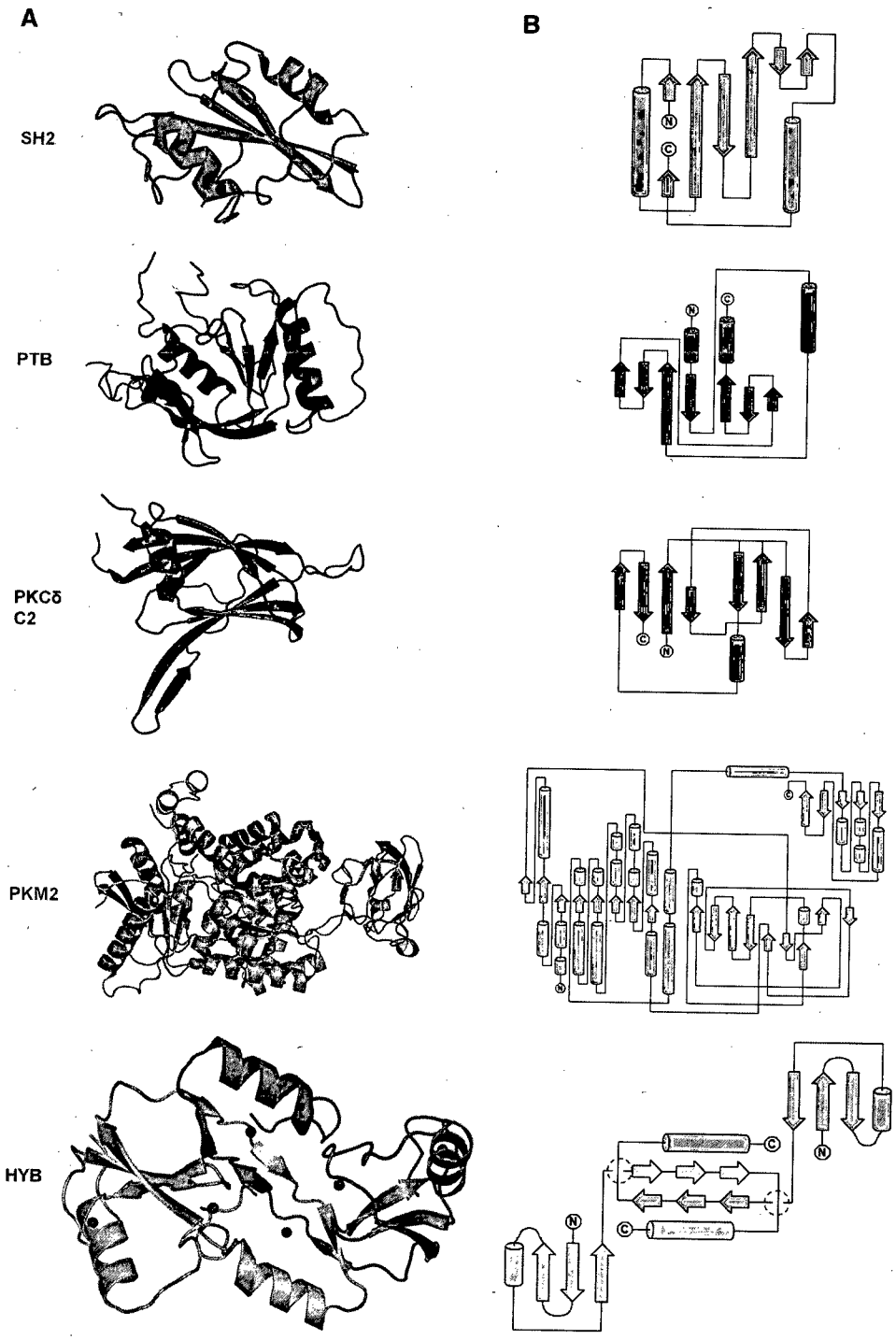
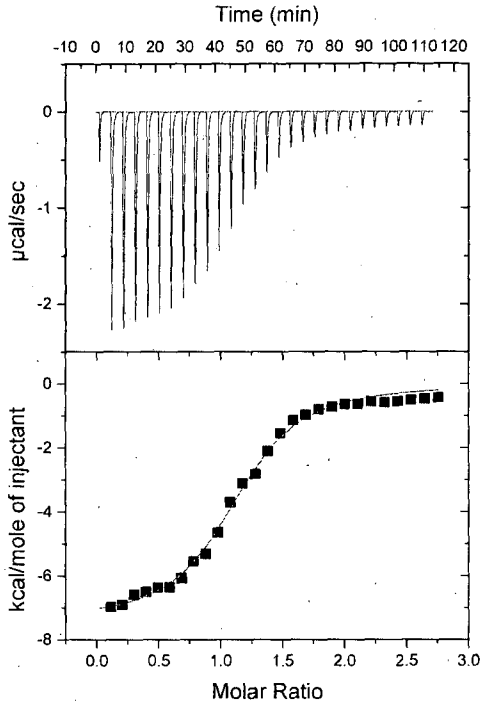
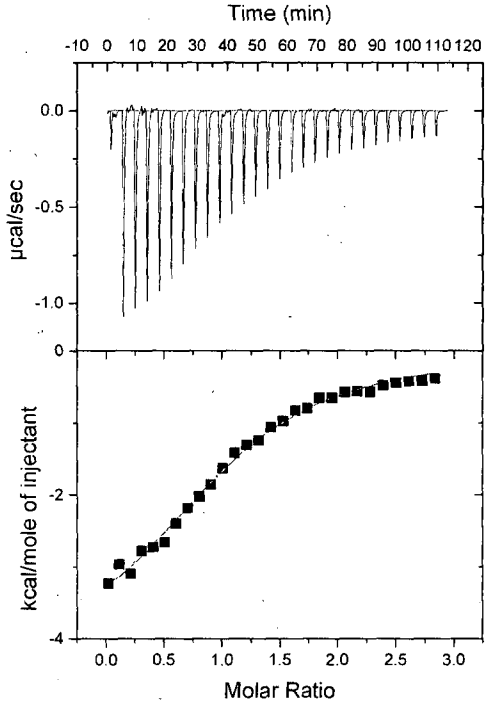


Figure 8

A. Hakai (106-206) binds with DOK1



B. Hakai (106-206) binds with cortactin



Peptide	Sequence	N	K_a(x10^6 M^{-1})	K_d(µM)	ΔH(kcal/mol)	TΔS(kcal/mol)	ΔG(kcal/mol)
DOK1$^{356-366}$	KEDPIpYDEPEG	1.159±0.020	0.1873±0.026	5.33	-7.41±0.187	-0.22	-7.19
Cortactin$^{477-489}$	AEDDTpYDGpYESDL	0.975±0.026	0.0351±0.003	28.4	-4.847±0.0189	1.33	-6.17

Figure 9

>Hakai sequence

MDHTDNELQGTNSSGSLGGLDVRRRIPIKLISKQASKVKPAPRTQRTVSRMPAKAPQGDE
EGFDYNEEQRYDCKGGELFGNQRRFPGHLFWDFKINILGEKDDTPVHFCDKCGLPIKVYG
RMIPCKHVFCYDCAILHEKKGDKMCPGCSDPVQRIEQCTRGSLFMCSIVQGCKRTYLSQR
DLQAHINHRHMRAGKPVTRASLENVHPPIAPPPTDIPDRFIMPPDKHHMSHIPPKQHIMM
PPPPLQHVPHEHYNQPHEDIRAPPAELSMAPPPPRSVSQETFRISTRKHSNLITVPIQDD
SSSGAREPPPPAPAPAHHHPEYQGQPVVSHPHHIMPPQQHYAPPPPPPPPISHPMPHPPQ
AAGTPHLVYSQAPPPPMTSAPPPITPPPGHIIAQMPPYMNHPPPGPPPPQHGGPPVTAPP
PHHYNPNSLPQFTEDQGTLSPPFTQPGGMSPGIWPAPRGPPPPPRMQGPPSQTPLPGPHH
PDQTRYRPYYQ

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2012/000487

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/50 (2006.01) C07K 14/00 (2006.01) C12N 9/12 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS. MEDLINE. HCAPLUS. EPODOC. WPIDS:

Searched terms including HAKAI, CBLLl, RNF188, E-CADHERIN BINDING PROTEIN E7, CAS-BR-M ECOTROPIC RETROVIRAL TRANSFORMING SEQUENCE, ZNF645, LIGAND OF NUMB PROTEIN, LNX1, LNX2, DOMAIN, INTERACTION, SITE, RECOGNITION, MOTIF, REGION, PHOSPHOTYROSINE, PTB, PI DOMAIN, DFMER, HOMODIMER, METHOTREXATE HYDRATE and CANCER together with like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2013	26 March 2013

Name and mailing address of the ISA/All

AUSTRALIAN PATENT OFFICE
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Authorised officer

Rebecca Hinton
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. 0262832194

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/064773 A2 (CHIRON CORPORATION et al.) 05 August 2004 Description - para [07] p2, para [40]-[47] pi 1-15; Sequence listing, SEQ ID NO:2.	1, 2, 5, 6, 9-18
X	Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, Behrens J, Sommer T, Birchmeier W. Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. Nat Cell Biol. 2002 Mar;4(3):222-3 1. Entire 'Results' section p222-229; Figure 3a p224.	1, 3, 7, 19
X	WO 2006/014706 A2 (SERENEX, INC et al.) 09 February 2006 Whole document especially abstract, claims	20-22

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-19 and 23 are directed to a method of drug screening using polypeptide sequences which characterise a novel phosphotyrosine binding domain (HYB), together with the isolated polypeptide sequences themselves and a crystal form of the isolated peptides.. The feature of the novel phosphotyrosine binding domain (HYB) sequence/structure is specific to this group of claims.

- Claims 20-22 are directed to the use of methotrexate hydrate (or a derivative or salt thereof) in providing treatment for a disease characterised by migration, metastasis, invasion or a lack of cell adhesion.. The feature of methotrexate hydrate (or a derivative or salt thereof) in providing a treatment for the said diseases is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori.*

The International Search Authority believes that a search and examination fee for the second invention will not involve more than a negligible additional search and examination effort over that for the first invention and thus all claims have been searched and examined for the purposes of the opinion.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2012/000487

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item l.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐ on paper

☒ in electronic form

b. (time)

☐ in the international application as filed

☒ together with the international application in electronic form

☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments :

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2012/000487

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos. :
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. Ill Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/SG2012/000487**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2004/064773 A2	05 Aug 2004	CA 2513182 A1	05 Aug 2004
		EP 1583826 A2	12 Oct 2005
		US 2005049214 A1	03 Mar 2005
		US 2006188989 A1	24 Aug 2006
		WO 2004064773 A2	05 Aug 2004
WO 2006/014706 A2	09 Feb 2006	US 2006035901 A1	16 Feb 2006
		WO 2006014706 A2	09 Feb 2006

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.