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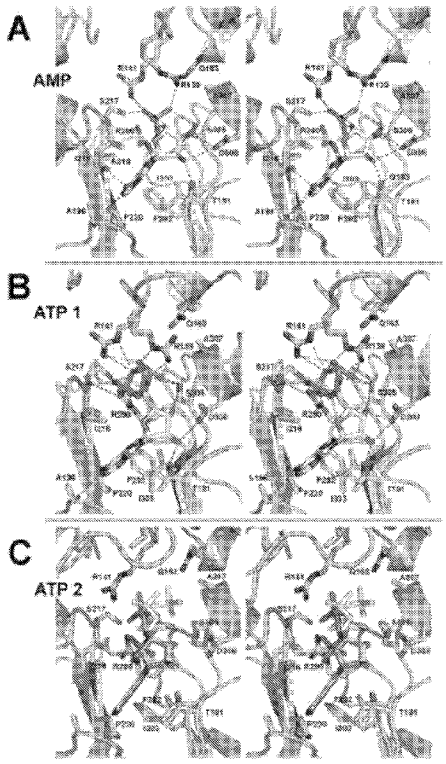

(57) Abstract: The teachings relate to the three-dimensional structures of crystals of an AMP kinase trimer core complexed with a ligand. An AMPK trimer core-ligand crystal structure, wherein the ligand is an agonist or an antagonist, provides structural information that can be integrated into drug screening and drug design processes. Thus, the teachings also relate to methods for utilizing a crystal structure of an AMPK-ligand complex for identifying, designing, selecting, or testing agonists and antagonists of an AMP kinase protein. Such inhibitors are useful as therapeutics for the prevention or treatment of diseases or disease symptoms, or for modifying other physiological events mediated by AMP kinase.

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CRYSTALS COMPRISING COMPLEXES OF REGULATORY DOMAINS OF AMP KINASE AND A LIGAND, AND METHODS OF USE THEREFOR

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to US Provisional patent application 60/867,036 filed November 22, 2006, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] The disclosed subject matter was developed in part with Government support under National Institutes of Health Grants GM-062270 and GM-30518. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED IN TEXT FILES

[0003] The accompanying lengthy table text files, filed herewith, are a part of the present disclosure and include files "TABLE_1_AMPK_AMP_coordinates.txt" having a size of 759 KB, the table having over 50 pages, comprising atomic coordinates exemplary of a crystal structure, and "TABLE_2_AMPK_ATP_coordinates.txt" having a size of 731 KB, the table having over 50 pages, comprising atomic coordinates exemplary of a crystal structure. A portion of the disclosure of this patent document contains material which may be subject to copyright protection. The copyright owner of that material has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure, as it appears in the Patent and Trademark Office patent files or records, but otherwise reserves all copyright. The subject matter of the lengthy text files are incorporated herein by reference in its entirety.

[0004] The Sequence Listing, which is a part of the present disclosure, includes a computer readable form and a written sequence listing comprising amino acid sequences of the present teachings. The sequence listing information recorded in computer readable form is identical to the written sequence listing. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND

FIELD

[0005] The disclosed subject matter relates to three dimensional structures and models of the regulatory domains of adenosine monophosphate-activated kinase complexed with various ligands, and uses thereof.

INTRODUCTION

[0006] Adenosine monophosphate-activated protein kinase (AMPK) is a master regulator of cellular metabolism. The 5'-AMP-activated protein kinase (AMPK), conserved in all eukaryotic organisms, coordinates metabolic function with energy availability (1-3). AMPK senses the onset of energy limitation and initiates adaptive responses including regulation of key enzymes in each of the major branches of metabolism, such as fatty acid synthesis , sugar metabolism, protein synthesis, and DNA replication (1-3). Long-term regulation by AMPK is effected by phosphorylation of transcription factor targets that regulate each of these metabolic functions (4). AMPK is activated by binding to adenosine monophosphate (AMP), but this activation is inhibited by adenosine triphosphate (ATP) (1-3). Because the intracellular ratio of adenosine diphosphate (ADP) to ATP remain nearly constant due to the equilibrium maintained by adenylate kinase, AMPK activity in vivo depends primarily on the AMP/ATP ratio, the primary determinant of cellular "energy charge," (5) defined as $\text{Energy charge} = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$. Lowered cellular energy charge can arise either from inhibition of ATP synthesis, for example in hypoxia or starvation (5), or by increased ATP consumption, as occurs in skeletal muscle during exercise (6, 7). When energy charge is low, active AMPK inhibits numerous ATP-consuming pathways, and also activates mobilization of intracellular energy stores to produce ATP. Conversely, when energy charge is high, AMPK inactivation is favored, leading to enhancement of energy storage and use. AMPK thus functions as a central switch that toggles cellular metabolism between energy storing (anabolic) and energy expending (catabolic) programs. AMPK also functions in organism-level energy homeostasis by responding to systemically-circulating hormones including leptin (8), adiponectin (9), and resistin (10). Through these mechanisms, AMPK plays a central role as a regulator of both organismal and cellular feeding behavior (11-14).

[0007] Activation of AMPK requires both a marked decrease in cellular ATP levels and phosphorylation by upstream kinases at amino acid Thr189 (following numbering for amino acid sequences of the yeast AMPK) in the activation loop of the α kinase domain (1-3). The primary upstream kinase is the tumor suppressor LKB1 (15, 16), but calmodulin-dependent protein kinases can also function in this role (17). The binding of AMP to AMPK promotes activation both by stimulating phosphorylation of the kinase domain, and inhibiting dephosphorylation of the activation loop threonine by protein phosphatases (18). These effects, in concert with the allosteric activation of the complex effected by AMP binding, are believed to collaborate in making the system exquisitely sensitive to small changes in cellular energy charge (9).

[0008] Canonical AMPKs are αβγ heterotrimers (1-3). Although gene fusions between the β and γ subunits are found in some plant species, the overall domain architecture of the enzyme is conserved in all eukaryotes (19). In humans multiple genes encode isoforms of each AMPK subunit (α1, α2, β1, β2, γ1, γ2, γ3) (1). The α subunit includes a typical serine/threonine kinase domain near the N-terminus, and a C-terminal regulatory domain characteristic of AMPKs. AMPK β subunits are required for trimer assembly and subcellular localization of the AMPK complex (1, 20). Many β isoforms include a glycogen-binding domain (GBD) for which structures are now known. β subunits can also be myristoylated, accounting for frequently observed membrane association of AMPKs (20, 21). Outside of the GBD, AMPK β subunits have no close sequence relatives. The heart of AMPK regulation by adenylate binding is thought to reside in γ subunits, which are composed of four repeated cystathionine β-synthase (CBS) domains (22-24). Although a number of CBS domain crystal structures have been determined (25-27), none of these includes a bound ligand. Biochemical and genetic studies have produced an initial view of interactions among AMPK subunits (1), whereby β subunits play a central role in heterotrimer formation through interactions with both α and γ subunits. Trimer interaction sites on both α and β subunits have been mapped to their respective C-terminal regions (28). Nonetheless, the mechanism by which AMPK is regulated remains unclear.

[0009] Among its activities, AMPK activity up-regulates import of glucose into a cell by glucose transporters. This effect is an important consideration for treating diabetes, as a primary problem in diabetes is elevated glucose in the blood, which is believed to be caused by an inability of cells to take up glucose. It is further believed that activated AMPK can phosphorylate some factors which stimulate pathways that lead to accumulation of glucose

transporters on a cell plasma membrane. Hence, diabetes treatments have been attempted which involve up-regulation of AMPK activity by artificially raising cellular AMP levels. These attempts have met with limited success.

[0010] The development of useful reagents for diagnosis, prevention or treatment of diseases such as diabetes has been hindered by lack of structural information on AMPK, and in particular complexes comprising an AMPK regulatory region and an AMPK ligand.

SUMMARY

[0011] The present inventors have developed crystals comprising complexes of regulatory domains of amp kinase and a ligand, as well as methods of their use. In view of the need for new reagents for diagnosis, prevention or treatment of diseases which involve AMPK, the present inventors have realized that there is a need to elucidate three dimensional structures and models of AMPK-ligand complexes, and to use such structures and models in therapeutic strategies, such as drug design. Furthermore, the present inventors realized that the regulatory region (i.e., the energy charge sensor region) of AMPK is a good candidate target for a drug which increases AMPK activity. They have, accordingly, devised methods for forming crystals comprising the regulatory region of AMP-activated protein kinase (an AMPK "trimer core") bound to a ligand, such as ATP or AMP, or a mimetic or analogue thereof.

[0012] To define a crystallizable AMPK trimer core for structural studies, the present inventors produced full-length human α2β1γ1 AMPK trimers by bacterial co-expression (see Examples). The inventors further developed methods of producing crystals comprising complexes of AMPK regulatory region, and an AMPK ligand. To produce these crystals, the inventors found that limited digests of an AMPK with proteases such as trypsin and/or lys-C, followed by size exclusion chromatography, can reveal a number of candidate fragments that retain heterotrimeric association. In some aspects of their discoveries, the inventors found that a recombinantly produced human α2β1γ1 trimer corresponding to one of these fragments can yield crystals that diffract x-rays to ~8 Å resolution.

[0013] In some aspects, the present teachings include methods of producing trimer core fragments from the AMPK gene of the yeast Schizosaccharomyces pombe corresponding to the human α2β1γ1 AMPK trimer cores. In some configurations of the present teachings, a fragment can yield two distinct crystal forms, depending upon the AMPK ligand: in some aspects, a crystal form comprising an S. pombe αβγ AMPK trimer core

complexed with the AMPK ligand AMP (the "AMP complex") can yield an AMPK trimer core-ligand complex having a three-dimensional structure which can be resolved by x-ray crystallography to about 2.6 Å resolution, while in other aspects, a crystal form comprising an S. pombe αβγ AMPK trimer core complexed with the AMPK ligand ATP (the "ATP complex") can yield an AMPK trimer core-ligand complex having a three-dimensional structure which can be resolved by x-ray crystallography to about 2.9 Å resolution. The trimer core in these crystals can include peptide fragments consisting of amino residues α 440-576 and β 205-298, as well as the entire γ subunit 1-334 (Fig. 1). In various configurations, such trimer cores can omit two regions of AMPK: the N-terminal glycogen binding domain of the β subunit, and the N-terminal kinase domain of α subunit. In various aspects of the present teachings, significant sequence conservation among AMPKs among different species indicates that the structures presented here are representative of AMPKs from a wide variety of species, including eukaryotic microorganisms such as yeast, plants, and animals including vertebrates such as mammals, including humans.

[0014] Accordingly, the present inventors have developed methods for designing a drug which enhances AMPK activity. These methods, in various configurations, comprise providing on a digital computer a three-dimensional structure of an AMPK trimer core-ligand complex comprising the trimer core of an AMPK and an AMPK ligand; and using software comprised by the digital computer to design a chemical compound which is predicted to bind to the AMPK trimer core.

[0015] In other aspects, the present inventors have developed methods for designing a drug which inhibits activity of an adenosine monophosphate-activated kinase (AMPK). In various configurations, these methods comprise providing on a digital computer a three-dimensional structure of an AMPK trimer core-ligand complex comprising the trimer core of an AMPK and an AMPK ligand; and using software comprised by the digital computer to design a chemical compound which is predicted to bind to the AMPK trimer core.

[0016] In some configurations, the AMPK trimer core can consist of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3. In some configurations, the AMPK trimer core can consist essentially of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3. In some configurations, the AMPK trimer core can comprise amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3. Hence, in various aspects, the methods can further comprise

synthesizing or obtaining the chemical compound; and evaluating the chemical compound for an ability to enhance or antagonize activity of the AMPK.

[0017] In various configurations of the present teachings, an AMPK trimer core which can be used to form a complex with an AMPK ligand which can be crystallized can include an AMPK trimer core obtained from any AMPK known to skilled artisans, such as, for example α, β, and γ chains of an AMPK of a eukaryotic microbe, an AMPK of a plant, or an AMPK of an animal, such as a vertebrate animal. In various aspects, an AMPK of a eukaryotic microbe can be an AMPK of a yeast such as Saccharomyces cerevisiae or Schizosaccharomyces pombe, and an AMPK of a vertebrate animal can be an AMPK of a mammal such as, for example, a human AMPK or a pig AMPK. In some configurations, an AMPK can be a hybrid or mosaic AMPK, such as, for example, a "humanized" S. pombe AMPK, in which one or more regions of a yeast trimer core are substituted with homologous regions of a human AMPK trimer core. In some configurations, a trimer core can include polypeptide chains or subsequences thereof from more than one species source; for example α and β subunits from a human AMPK, and a γ subunit from a yeast AMPK, or a substitution of a subsequence of part of a yeast AMPK polypeptide with a homologous sequence from a human polypeptide. In some aspects, an AMPK trimer core can include amino acid substitutions additions, or deletions, such as conservative substitutions, provided the trimer core retains the ability to bind at least one AMPK ligand such as AMP, ATP, or a mimetic or analogue thereof. In addition, in various configurations, a polypeptide comprising a trimer core and which binds at least one AMPK ligand can diverge in sequence from a homologous sequence of a naturally occurring AMPK such as a human or a yeast AMPK, but retain at least about 70% sequence identity, at least about 80% sequence identity, at least about 90% sequence identity with a wild type AMPK polypeptide, or at least about 95% sequence identity with the homologous sequence. In some configurations, a trimer core can comprise an α subunit fragment from S. pombe, consisting of or consisting essentially of amino acids 440-576 (SQSTRKKSRRNKWHFGVRCRGDAPEILLAVYRALQRAGAQFTVPKPVNGKYRSDMYTIKSRWEIPHCKREGKNTYAYIELQLEVMPGCFMLDVKSNGYKDIYSHPERTADHGMDDLKSSFPFLDLCAMLVCKLFSA, SEQ ID NO: 1); a β subunit fragment from S. pombe, consisting of or consisting essentially of amino acids 205-298 (SEQYSTEIPAFLTSNTLQELKLPKPPSLPPHLEKCILNSNTAYKEDQSVLPNPNHVLLNHLAAANTQLGVLALSATTRYHRKYVTTAMFKNFDV, SEQ ID NO: 2) , and a γ subunit

from S. pombe, comprising amino acids 1-334 (MTDVQETQKGALKEIQAFIRSRTSYDVLPTSFRLIVFDVTLFVKTSLSLLTLNNIVSAP LWDSEANKFAGLLTMADFVNVIKYYYQSSSFPEAIAEIDKFRLLGLREVERKIGAIPPE TIYVHPMHSLMDACLAMSKSRARRIPLIDVDGETGSEMIVSVLTQYRILKFISMNCKE TAMLRVPLNQMTIGTWSNLATASMETKVYDVIKMLAEKNISAVPIVNSEGTLLNVY ESVDVMHLIQDGDYSNLDLSVGEALLKRPANFDGVHTCRATDRLDGIFDAIKHSRVH RLFVVDENLKLEGILSLADILNYIIYDKTTTPGVPEQTDNFESAV, SEQ ID NO: 3).

[0018] In various configurations of the present teachings, an AMPK ligand comprised by a crystal can be a nucleoside phosphate such as AMP or ATP, or a structural analog or mimetic thereof, for example an AMP analogue or mimetic such as 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (AICAR-phosphate, also called ZMP).

[0019] In various aspects, a chemical compound which can be a candidate agonist or antagonist of AMPK activity can be designed by computational interaction with reference to a three dimensional site of the structure of the AMPK-ligand complex, wherein the three dimensional site comprises one or more amino acids comprising a ligand binding site. For example, with reference to a S. pombe AMPK, a ligand binding site can comprise amino acids A196, A218, R290, S217, R141, R139, D308, S305, T191 (amino acids contacting AMP and/or ATP) or a combination thereof.

[0020] In certain aspects of the present teachings, the present inventors have developed methods for generating a model of a three dimensional structure of a target AMPK or a trimer core thereof. In these aspects, a method can comprise (a) providing amino acid sequences of the polypeptides of a reference AMPK or trimer core thereof, and amino acid sequences of a target AMPK, or a trimer core thereof which is comprised by an AMPK trimer core-ligand complex; (b) identifying structurally conserved regions shared between the reference AMPK or trimer core thereof and the target AMPK or trimer core thereof; and (c) assigning atomic coordinates to the target AMPK trimer core-ligand complex from the conserved regions. The amino acid sequences of AMPK polypeptides or a trimer core thereof in these aspects can be as described above. In these aspects, a target AMPK trimer core-ligand complex can have a three dimensional structure described by atomic coordinates which substantially conform to atomic coordinates set forth in Table 1 (describing coordinates of an AMPK trimer core complexed with AMP) or in Table 2 (describing coordinates of an AMPK trimer core complexed with ATP). In addition, a target AMPK

trimer core-ligand complex can have a three dimensional structure described by atomic coordinates of an AMPK trimer core complexed with 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (AICAR-phosphate, also called ZMP).

[0021] In certain aspects of the present teachings, the present inventors have developed methods for determining a three dimensional structure of a target AMPK trimer core-ligand complex. In various configurations of these aspects, these methods comprise (a) providing amino acid sequences of polypeptide chains comprising a target AMPK or a trimer core thereof; (b) predicting the pattern of folding of the polypeptide chains in a three dimensional conformation using a fold recognition algorithm; and (c) comparing the pattern of folding of the target structure amino acid sequences with the three dimensional structure of a known reference AMPK trimer core-ligand complex. In various aspects, the amino acid sequences of a target AMPK can be as described above. In various aspects, a reference AMPK trimer core-ligand complex can comprise a three dimensional structure described by atomic coordinates that substantially conform to atomic coordinates of an S. pombe AMPK trimer core as set forth in Table 1 or Table 2.

[0022] The present teachings include methods for increasing AMPK activity. In various configurations, these methods include selecting an AMPK agonist by performing a structure based drug design using a three-dimensional structure determined for a crystal comprising a trimer core of an AMPK and an AMPK ligand, and contacting a sample comprising AMPK with the agonist. The AMPK, in various aspects, can be comprised by a subject such as a human patient in need of treatment with an AMPK agonist.

[0023] The present teachings also include methods for decreasing AMPK activity. In various configurations, these methods include selecting an AMPK antagonist by performing a structure based drug design using a three-dimensional structure determined for a crystal comprising a trimer core of an AMPK and an AMPK ligand, and contacting a sample comprising an AMPK with the antagonist. The AMPK, in various aspects, can be comprised by a subject such as a human patient in need of treatment with an AMPK antagonist. In these configurations, an AMPK trimer core and an AMPK ligand can be those described above.

[0024] In various aspects of the present teachings, the present inventors disclose a crystal comprising an AMPK trimer core and an AMPK ligand. In certain configurations of these aspects, an AMPK ligand can be a nucleotide such as AMP, and a crystal can be sufficiently ordered for resolving at structure at about 2.6 Å resolution, while in other

configurations an AMPK ligand can be a nucleotide such as ATP, and a crystal can be sufficiently ordered for resolving at structure at about 2.9 Å resolution. A crystal comprising an AMPK trimer core-complex with ATP or AMP can comprise a space group $P2_12_12_1$ so as to form a unit cell of dimensions a~73.5 Å, b~97.4 Å, c=168.9 Å. In an alternative configuration, a crystal comprising an AMPK trimer core complex with ATP or AMP can comprise a space group $P2_1$ so as to form a unit cell of dimensions a~46.2 Å, b~39.9 Å, c=66.5 Å, and β=110.7°. In various aspects, a complex of an AMPK trimer core and a nucleotide ligand can include contacts between the trimer core and the ligand at one or more amino acids selected from the group consisting of A196, A218, R290, S217, R141, R139, D308, S305, T191 (amino acids contacting AMP and/or ATP).

[0025] In some aspects, the present teachings include a therapeutic compound which enhances AMPK activity. Such compounds can cause a reduction in serum glucose levels when administered to a subject by stimulating cellular glucose uptake by cells contacting the compound. In these aspects, a compound can be selected by a) performing a structure based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, b) contacting a sample comprising AMPK with the compound. In some configurations, the method can further include c) detecting enhancement of at least one activity of the AMPK. In some aspects, the activity of the AMPK can be activation of glucose transporters.

[0026] In some aspects, the present teachings include a therapeutic compound which inhibits AMPK activity. In these aspects, a compound can be selected by a) performing a structure based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, b) contacting a sample comprising AMPK with the compound. In some configurations, the method can further include c) detecting inhibition of at least one activity of the AMPK. In some aspects, the activity of the AMPK can be inactivation of glucose transporters.

[0027] Certain aspects of the present teachings include a three dimensional computer image of the three dimensional structure of an AMPK trimer core-ligand complex. In these aspects, a structure can substantially conform with the three dimensional coordinates listed in Table 1 or Table 2.

[0028] Certain aspects of the present teachings include a computer-readable medium encoded with a set of three dimensional coordinates as set forth in Table 1 or Table 2. In

these aspects, the three dimensional coordinates set forth in Table 1 or Table 2 can be used in conjunction with a graphical display software program to create an electronic file that can be visualized on a computer capable of representing the electronic file as a three dimensional image.

[0029] Certain aspects of the present teachings include a computer-readable medium encoded with a set of three dimensional coordinates of a three dimensional structure which substantially conforms to the three dimensional coordinates represented in Table 1 or Table 2. In these aspects, using a graphical display software program, the set of three dimensional coordinates can be used to create an electronic file that can be visualized on a computer capable of representing the electronic file as a three dimensional image.

[0030] Some aspects of the present teachings disclose methods of forming a crystal comprising an AMPK trimer core and an AMPK ligand. In various configurations, these methods can comprise forming a composition comprising the AMPK trimer core, the ligand, water; adding a solution comprising a polyethylene glycol, Ethylene Glycol, and a buffer. The trimer core can be a trimer core as described herein, and the ligand can be a nucleotide such as AMP, ADP or ATP, or an analogue or mimetic thereof.

[0031] The present inventors also set forth methods for elevating AMPK activity in a subject for the treatment of a disease. Such methods comprise: selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof. In some aspects, the disease can be diabetes, such as type 2 diabetes.

[0032] Also disclosed herein are methods for stimulating glucose uptake in a cell or tissue. These methods comprise: selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and contacting the cell or tissue with an effective amount of the agonist. In various aspects, the cell or tissue can be a muscle cell or muscle tissue.

[0033] The present teachings also include methods of achieving whole-body glycemic control. These methods comprise, in various embodiments, selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for

a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0034] Various aspects of the present teachings include methods of decreasing glucose production in a liver cell or tissue. These methods can comprise: selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0035] Yet other aspects of the present teachings include methods of decreasing lipid synthesis in a liver cell or tissue. These methods can comprise selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0036] In further aspects, the present teachings disclose methods of increasing lipid oxidation in a liver cell or tissue. In various configurations, these methods comprise: selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0037] Yet other aspects of the present teachings include methods of decreasing lipolysis in a cell or tissue. These methods comprise selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0038] In some configurations, such methods can be used to decrease lipolysis in adipose cells or tissue.

[0039] In yet another aspect, the present teachings include methods of decreasing lipogenesis in a cell or tissue. These methods can comprise selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the agonist to a subject in need thereof. In these aspects, the cells or tissue can be an adipose cell or tissue.

[0040] In additional aspects, the present teachings include methods of decreasing circulating lipid levels. These methods can comprise selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the agonist to a subject in need thereof. In these aspects, the cells or tissue affected by the AMPK agonist can include adipose cells or tissue.

[0041] In yet other aspects, the inventors provide methods of decreasing ectopic fat deposition. These methods can comprise: selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the agonist to a subject in need thereof. The cells or tissue can be adipose cells or tissue. In various configurations, these methods can comprise selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the agonist to a subject in need thereof.

[0042] In yet other aspects, the present teachings include methods of promoting longevity. In some aspects, the methods comprise administering to a subject an agonist of AMPK. In some aspects, these methods can further comprise selecting an AMPK agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising a trimer core of an AMPK and an AMPK ligand.

[0043] The present teachings also provide that selecting an AMPK agonist can comprise a) identifying a candidate agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal of a complex comprising an AMPK trimer core and an AMPK ligand; b) contacting the candidate agonist with an AMPK; and c) detecting enhancement of at least one activity of the AMPK. In some configurations, the AMPK can be a human AMPK, and the AMPK trimer core can be a yeast AMPK trimer core.

[0044] In yet other aspects, the present teachings include methods for decreasing AMPK activity in a subject for the treatment of a disease. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an

AMPK ligand, and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0045] Furthermore, some aspects of the present teachings include methods for inhibiting glucose uptake in a cell or tissue. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK. These methods can include: selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0046] Some aspects of the disclosure include methods of increasing lipid synthesis in a liver cells or tissue. These methods can comprise: selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0047] The present methods also include methods of decreasing lipid oxidation in liver cells or tissue. These methods can comprise: selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0048] In some configurations, the present teachings present methods of increasing lipolysis in cells or tissues. These methods include selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0049] In some aspects of these methods, the cells or tissue can be adipose cells or tissue.

[0050] Yet other aspects include methods of increasing lipogenesis in cells or tissue. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective

amount of the antagonist to a subject in need thereof. In these aspects, the cells or tissue can be adipose cells or tissue.

[0051] The present inventors also provide methods of increasing circulating lipid levels. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the antagonist to a subject in need thereof. In these methods, the cells or tissue can be adipose cells or tissue.

[0052] Similarly, some aspects include methods of increasing ectopic fat deposition. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and administering a therapeutically effective amount of the antagonist to a subject in need thereof. In these methods, the cells or tissue can be adipose cells or tissue.

[0053] Some aspects of the present teachings include methods of increasing insulin secretion by the pancreas. These methods can comprise selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand, and administering a therapeutically effective amount of the antagonist to a subject in need thereof.

[0054] In various configurations, the methods which include selecting an antagonist can comprise a) identifying a candidate agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal of a complex comprising an AMPK trimer core and an AMPK ligand, b) contacting the candidate antagonist with an AMPK, and c) detecting inhibition of at least one activity of the AMPK.

[0055] In these configurations, the AMPK can be a human AMPK, and furthermore, the AMPK trimer core can be a yeast AMPK trimer core such as an S. pombe AMPK trimer core.

[0056] Also provided are high throughput screening methods for identification of AMPK agonists and/or antagonists. Such methods include the steps of selecting a candidate compound; providing an AMPK trimer core and an AMPK kinase domain; contacting the candidate compound, the AMPK trimer core, and the AMPK kinase domain; detecting association and/or dissociation of the AMPK trimer core and the AMPK kinase domain; and

correlating association and/or dissociation of the AMPK trimer core and an AMPK kinase domain with agonist or antagonist activity, respectively, of the candidate compound. In some configurations, an AMPK is further provided for contact with the candidate compound, the AMPK trimer core, and the AMPK kinase domain. In some configurations, selection of a candidate compound involves performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand.

[0057] These and other features, aspects and advantages of the present teachings will become better understood with reference to the following description, examples, and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0059] FIG. 1 depicts the overall structure of the adenylate binding region from S. pombe AMPK with bound AMP. The ATP-bound form is nearly identical (see superposition in Fig. 10), and reveals no global structural changes attributable to nucleotide identity. (A) Ribbon diagram of a single heterotrimer, with the α, β, and γ subunits originally colored yellow, blue and green, respectively. The single molecule of bound AMP is shown in CPK representation, and connections to the glycogen binding domain (GBD) and kinase domain (KD) at the N-termini of the β and α subunits, respectively, are indicated. (B) View rotated 90°, highlighting the nucleotide binding face (AXP), and phosphate binding tunnel, which is capped on the putative kinase domain-binding surface by a polar flap from the β subunit. The structure corresponds to a heterotrimer defined by limited proteolysis, as indicated in (C). Each of the two crystal forms reported here includes a dimer of trimers in the asymmetric unit (D). Analytical ultracentrifugation analysis also demonstrates a dimer of trimers configuration. Colors of the original color figure are displayed individually, superimposed upon a dimmed original image as a whole.

[0060] FIG. 2 depicts nucleotide binding. (A) Stereo diagram of AMP bound within the γ subunit. Adenine and ribose moieties are bound by functional groups within the CBS3/4 domain pair, however the terminal α-phosphate forms salt bridges with two Arg side chains (R139 and R141) donated from CBS domain 2. (B) ATP binds through a set of identical

protein ligands, accommodating the β and γ phosphates of ATP by adopting a compact helical structure for the triphosphate group. This electrostatically unfavorable conformation is likely key to the favored binding of AMP, important to the tuned sensor function of AMPK. (C) The ATP binding site of second molecule in the asymmetric unit of the ATP complex structure reveals at least two alternative phosphate conformations, and density is not observed for the side chain of R139. Despite the topological identity of the nucleotide-binding CBS3/4 (originally yellow) domain pair and the non-binding CBS1/2 pair (originally blue) (D), the CBS1/2 pair lacks features required for nucleotide binding (E). Superpositions including the bound nucleotide of CBS3/4 show that the separation between β-strands in CBS1/2 is too narrow to accommodate the nucleotide base, the polar and hydrophobic chemical character of the base and sugar binding pockets are not maintained, and the analogous phosphate-binding ligands from the other CBS domain pair would be positioned improperly to bind phosphates of a nucleotide analogously bound at CBS1/2. Electrostatic surfaces of the putative kinase-binding face shows that the charge potential of the exposed part of the phosphate tunnel is positive when AMP is bound (F), but negative when ATP is bound (G). Colors of the original color figure are displayed individually, superimposed upon a dimmed original image as a whole in panels 2D and 2E.

[0061] FIG. 3 depicts a functional mutations map within the phosphate binding tunnel, a large internal cavity that traverses the γ subunit, originally shown in red. The majority of known function-impairing mutants map to the surface of this tunnel, positioned between the terminal phosphate of the bound nucleotide and the putative kinase-binding face. Orthogonal views are shown in (A) and (B). The phosphate tunnel is capped on the putative kinase-binding face by a highly mobile polar flat descending from the β subunit, originally shown in gold (C). Colors of the original color figure are displayed individually, superimposed upon a dimmed original image as a whole.

[0062] FIG. 4 depicts biophysical interaction analysis and a schematic model for AMPK regulation by adenylate binding. (A) SPR experiments reveal binding between human AMPK α2 kinase domain (stationary phase) and the human heterotrimeric adenylate sensor (mobile phase) in the presence of AMP, and in the absence of nucleotide. However, no binding between kinase and sensor domains is found in the presence of ATP. The minimal kinase domain proper (residues 1-299) was used in these experiments, showing that sequences outside this region are not necessary for nucleotide-dependent interaction with the regulatory trimer. (B) Analytical ultracentrifugation analysis of the interaction between S.

pombe regulatory heterotrimer and the human AMPK α2 kinase domain, which is 59% identical to the corresponding region from the S. pombe protein. As for the SPR analyses of human AMPK, a 1:1 complex is formed between regulatory and catalytic regions in the absence of nucleotide or presence of AMP, but this complex does not form in the presence of ATP. Schematic models of ATP mediated inhibition based on the crystal structures and interaction analyses reported here are shown in (C-F). When AMP is bound (C), the phosphate tunnel has a positive electrostatic potential, and the kinase domain (orb) associates with the heterotrimer. Binding is also observed in biophysical measurements when no nucleotide is present. When ATP binds the regulatory trimer (D), the electrostatic potential of the phosphate tunnel is negative, and no binding is observed between the regulatory trimer and the kinase domain. In yeast (E and F), the regulatory trimer forms a dimer-of-trimers, and the kinase domain forms an auto-inhibited dimer, with active sites occluded. This dimer must dissociate, exposing the active sites in the presence of AMP. This is likely accomplished by the observed binding to the regulatory heterotrimers as shown in (E). When ATP is bound to the regulatory trimer, the kinase domains do not bind the regulatory trimer, allowing the kinase domain dimer to form, inhibiting AMPK activity (F).

[0063] FIG. 5 depicts the structure of the kinase-associated domain of the α subunit. Ribbon and topology diagrams (panel 5A), and a sequence alignment with AMPK α subunits from pig (α2) and human (α 1 and α 2) (panel 5B) are shown. The ribbon diagram maps the sequence from N- to C-terminus. Sequence conservation in the C-terminal kinase-associated domain region suggests that each AMPK family member will adopt a similar structure.

[0064] FIG. 6 depicts the structure of the β subunit. A ribbon diagram which maps the sequence from the N terminus to the C-terminus is shown in panel 6A. A sequence alignment with AMPK β subunits from S. cerevisiae, mouse (β1,) and human (β1) is shown in panel 6B. Sequence conservation suggests that each AMPK family member will adopt a similar structure.

[0065] FIG. 7 depicts the structure of the γ subunit. Ribbon and topology diagrams are shown above, and a sequence alignment with AMPK γ subunits from S. cerevisiae, human (γ1), and pig (γ1) and are shown in panel 7A and panel. 7B, respectively. The ribbon diagram maps the sequence from the N-terminus to the C-terminus. Sequence conservation suggests that each AMPK family member will adopt a similar structure.

[0066] FIG. 8 is a detailed stereo view of the α/β interface. The α subunit (yellow in original), and the β subunit (blue in original) are shown. Side chains are drawn for all residues that bury at least 30% of their molecular surface are in the α/β interface, which buries ~2300 $Å^2$ of total molecular surface area. Colors of the original color figure are displayed individually, superimposed upon a dimmed original image as a whole.

[0067] FIG. 9 is a detailed stereo view of the β/γ interface. All three subunits are shown: α (yellow in original), β subunit (blue in original), and γ (green in original). Bound AMP is shown in CPK representation. Side chains are drawn for all residues that bury at least 30% of their molecular surface are in the β/γ interface, which buries ~1300 $Å^2$ of total molecular surface area. Colors of the original color figure are displayed individually, superimposed upon a dimmed original image as a whole.

[0068] FIG. 10 is a stereo diagram of superposed Cα traces of the AMP- (dark) and ATP-(light gray) bound AMPK regulatory heterotrimer complexes. Bounds AMP and ATP ligands are also are also shown in dark and light gray, respectively. No significant shifts attributable to binding of the different adenylate ligands are observed. The RMS coordinate deviation is 0.90 Å for all α carbons.

[0069] FIG. 11 is a diagram depicting a FRET-based high throughput screening assay for AMPK agonists and/or antagonists.

DETAILED DESCRIPTION

[0070] The present teachings relate to the discovery of three-dimensional structures of an AMPK trimer core complexed with various ligands (each complex individually referred to as a "AMPK- trimer core ligand complex"), models of such three-dimensional structures, a method of structure-based drug design using such structures, the compounds identified by such methods and the use of such compounds in therapeutic compositions. In particular, the present teachings relate to crystals of AMPK trimer core complexed with ligands such as AMP or ATP, methods of production of such crystals; three dimensional coordinates of such complexes; three dimensional structures of the complexes; and uses of such structures and models to derive other AMPK trimer core-ligand complex structures and in drug design strategies.

[0071] One aspect of the present teachings includes models of AMPK trimer core-ligand complexes in which the models represent three dimensional structures of an AMPK trimer core-ligand complexes. Another aspect of the present teachings includes the three

dimensional structure of AMPK trimer core-ligand complexes, such as the three dimensional structure of an AMPK trimer core-ligand complex which substantially conforms with the atomic coordinates represented in Table 1 and/or Table 2, corresponding to an AMPK trimer core-AMP complex or an AMPK trimer core-ATP complex, respectfully. In the present teachings, the term "substantially conforms" refers to at least a portion of a three dimensional structure of an AMPK trimer core-ligand complex which is sufficiently spatially similar to at least a portion of a specified three dimensional configuration of a particular set of atomic coordinates (e.g., those represented by Table 1 and/or Table 2) to allow the three dimensional structure of an AMPK trimer core-ligand complex to be modeled or calculated using the particular set of atomic coordinates as a basis for determining the atomic coordinates defining the three dimensional configuration of an AMPK trimer core-ligand complex.

[0072] More particularly, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein at least about 50% of such structure has an average root-mean-square deviation (RMSD) of less than about 1.8 Å for the backbone atoms in secondary structure elements in each domain, and in various aspects, less than about 1.25 Å for the backbone atoms in secondary structure elements in each domain, and, in various aspects less than about 1.0 Å for the backbone atoms in secondary structure elements in each domain. In one aspect of the present teachings, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein at least about 75% of such structure has the recited average RMSD value, and in some aspects, at least about 90% of such structure has the recited average RMSD value, and in some aspects, about 100% of such structure has the recited average RMSD value. In particular, the above definition of "substantially conforms" can be extended to include atoms of amino acid side chains. As used herein, the phrase "common amino acid side chains" refers to amino acid side chains that are common to both the structure which substantially conforms to a given set of atomic coordinates and the structure that is actually represented by such atomic coordinates.

[0073] In another aspect of the present teachings, a three dimensional structure that substantially conforms to a given set of atomic coordinates is a structure wherein at least about 50% of the common amino acid side chains have an average RMSD of less than about 1.8 Å, and in various aspects, less than about 1.25 Å, and, in other aspects, less than about 1.0 Å. In one aspect of the present teachings, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein at least about 75% of the common amino acid side chains have the recited average RMSD value, and in some aspects, at least about 90% of

the common amino acid side chains have the recited average RMSD value, and in some aspects, about 100% of the common amino acid side chains have the recited average RMSD value.

[0074] A three dimensional structure of an AMPK trimer core-ligand complex which substantially conforms to a specified set of atomic coordinates can be modeled by a suitable modeling computer program such as MODELER (A. Sali and T.L. Blundell, J. Mol. Biol., vol. 234:779-815, 1993 as implemented in the Insight II software package Insight II, available from Accelerys (San Diego, Calif.)) and those software packages listed in the Examples, using information, for example, derived from the following data: (1) the amino acid sequence of the AMPK trimer core-ligand complex; (2) the amino acid sequence of the related portion(s) of the protein represented by the specified set of atomic coordinates having a three dimensional configuration; and, (3) the atomic coordinates of the specified three dimensional configuration. A three dimensional structure of an AMPK trimer core-ligand complex which substantially conforms to a specified set of atomic coordinates can also be calculated by a method such as molecular replacement, which is described in detail below.

[0075] A suitable three dimensional structure of the AMPK trimer core-ligand complex for use in modeling or calculating the three dimensional structure of another AMPK trimer core-ligand complex comprises the set of atomic coordinates represented in Table 1 and/or Table 2. The set of three dimensional coordinates set forth in Table 1 and/or Table 2 are represented in standard Protein Data Bank format. According to the present teachings, an AMPK trimer core-ligand complex has a three dimensional structure which substantially conforms to the set of atomic coordinates represented by Table 1 and/or Table 2. As used herein, a three dimensional structure can also be a most probable, or significant, fit with a set of atomic coordinates. According to the present teachings, a most probable or significant fit refers to the fit that a particular AMPK trimer core-ligand complex has with a set of atomic coordinates derived from that particular AMPK trimer core-ligand complex. Such atomic coordinates can be derived, for example, from the crystal structure of the protein such as the coordinates determined for the AMPK trimer core-ligand complex structure provided herein, or from a model of the structure of the protein. For example, the three dimensional structure of a monomeric or multimeric protein, including a naturally occurring or recombinantly produced AMPK trimer core, substantially conforms to and is a most probable fit, or significant fit, with the atomic coordinates of Table 1 and/or Table 2. The three dimensional crystal structure of the AMPK trimer core-ligand complex may comprise the atomic

coordinates of Table 1 and/or Table 2. Also as an example, the three dimensional structure of another AMPK trimer core-ligand complex would be understood by one of skill in the art to substantially conform to the atomic coordinates of Table 1 and/or Table 2. This definition can be applied to the other AMPK trimer cores in a similar manner.

[0076] In various aspects of the present teachings, a structure of an AMPK trimer core-ligand complex substantially conforms to the atomic coordinates represented in Table 1 and/or Table 2. Such values as listed in Table 1 and/or Table 2 can be interpreted by one of skill in the art. In other aspects, a three dimensional structure of an AMPK trimer core-ligand complex substantially conforms to the three dimensional coordinates represented in Table 1 and/or Table 2. In other aspects, a three dimensional structure of an AMPK trimer core-ligand complex is a most probable fit with the three dimensional coordinates represented in Table 1 and/or Table 2. Methods to determine a substantially conforming and probable fit are within the expertise of skill in the art and are described herein in the Examples section.

[0077] AN AMPK trimer core-ligand complex that has a three dimensional structure which substantially conforms to the atomic coordinates represented by Table 1 and/or Table 2 includes an AMPK trimer trimer of a protein having amino acid sequences that are at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 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%, 99%, or 100% identical to homologous amino acid sequences, such as amino acid sequences of AMPK trimer cores having sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, or AMPK polypeptides such as those set forth as SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14. Table 3 sets forth sequences of some AMPK polypeptides as well as sequences of the polypeptides comprised by crystals of the present teachings. A sequence alignment program such as BLAST (available from the National Institutes of Health Internet web site http://www.ncbi.nlm.nih.gov/BLAST) can be used by one of skill in the art to compare sequences of one AMPK or AMPK trimer corps to other AMPKs or AMPK trimer corps.

Table 3: Polypeptide sequences

Yeast S. pombe Alpha subunit amino acids 440-576	SQSTRKKSRR NKWHFGVRCR GDAPEILLAV YRALQRAGAQ FTVPKPVNGK YRSDMYTIKS RWEIPHCKRE GKNTYAYIEL QLEVMPGCFM LDVKSNGYKD IYSHPERTAD HGMDDLKSSF PFLDLCAMLV CKLFSA	SEQ ID NO: 1
Yeast S. pombe Beta subunit amino acids 205-298	SEQYSTEIPA FLTSNTLQEL KLPKPPSLPP HLEKCILNSN TAYKEDQSVL PNPNHVLLNH LAAANTQLGV LALSATTRYH RKYVTTAMFK NFDV	SEQ ID NO: 2
Yeast S. pombe Gamma subunit	MTDVQETQKG ALKEIQAFIR SRTSYDVLPT SFRLIVFDVT LFVKTSLSLL TLNNIVSAPL WDSEANKFAG LLTMADFVNV IKYYYQSSSF PEAIAEIDKF RLLGLREVER KIGAIPPETI YVHPMHSLMD ACLAMSKSRA RRIPLIDVDG ETGSEMIVSV LTQYRILKFI SMNCKETAML RVPLNQMTIG TWSNLATASM ETKVYDVIKM LAEKNISAVP IVNSEGTLLN VYESVDVMHL IQDGDYSNLD LSVGEALLKR PANFDGVHTC RATDRLDGIF DAIKHSRVHR LFVVDENLKL EGILSLADIL NYIIYDKTTT PGVPEQTDNF ESAV	SEQ ID NO: 3
Yeast S. pombe Alpha subunit	MQPQEVDLME NSTMRNGARV LPPEAISKRH IGPYIIRETL GEGSFGKVKL ATHYKTQQKV ALKFISRQLL KKSDMHMRVE REISYLKLLR HPHIIKLYDV ITTPTDIVMV IEYAGGELFD YIVEKKRMTE DEGRRFFQQI ICAIEYCHRH KIVHRDLKPE NLLLDDNLNV KIADFGLSNI MTDGNFLKTS CGSPNYAAPE VINGKLYAGP EVDVWSCGIV LYVMLVGRLP FDDEFIPNLF KKVNSCVYVM PDFLSPGAQS LIRRMIVADP MQRITIQEIR RDPWFNVNLP DYLRPMEEVQ GSYADSRIVS KLGEAMGFSE DYIVEALRSD	SEQ ID NO: 4

	ENNEVKEAYN LLHENQVIQE KSHLSKSKRV DSFLSVSPPA FSEYTSELQK KSKQELIDPT LEGPRWTVSD PPTYAKQTID SNICVLVPTA EKNKLEMRTL ADAASAVDTS QSTRKKSRRN KWHFGVRCRG DAPEILLAVY RALQRAGAQF TVPKPVNGKY RSDMYTIKSR WEIPHCKREG KNTYAYIELQ LYEVMPGCFM LDVKSNGYKD IYSHPERTAD HGMDDLKSSS PFSDLCAMLV CKLFSA	
Yeast S. pombe beta subunit	MGNVQSQEGE TRAHAVPSQD ATTTPDNANN VPKEPRAQSM ISIAADDLNQ EGEMSDDNQQ EGGNNRTSQN GTSGSSGHTK RRSQTSGKKT HQPYSGPCVP TIIRWRGGGE VVYVTGSFSR WKKKIQLLKS EDYTVLLQLR PGTQRFKFLV DGIWCCSSDF PTATDAEGNL YNYLEVEANE KLGASIDERL SQVHTDLPME EKSESEQYST EIPAFLTSNT LQELKLPKPP SLPPHLEKCI LNSNTAYKED QSVLPNPNHV LLNHLAAANT QLGVLALSAT TRYHRKYVTT AMFKNFDV	SEQ ID NO: 5
Pig alpha-2 subunit	MAEKQKHDGR VKIGHYVLGD TLGVGTFGKV KIGEHQLTGH KVAVKILNRQ KIRSLDVVGK IKREIQNLKL FRHPHIIKLY QVISTPTDFF MVMEYVSGGE LFDYICKHGR VEEMEARRLF QQILSAVDYC HRHMVVHRDL KPENVLLDAQ MNAKIADFGL SNMMSDGEFL RTSCGSPNYA APEVISGRLY AGPEVDIWSC GVILYALLCG TLPFDDEHVP TLFKKIRGGV FYIPEYLNRS VATLLMHMLQ VDPLKRATIK DIREHEWFKQ DLPSYLFPED PSYDANVIDD EAVKEVCEKF ECTESEVMNS LYSGDPQDQL AVAYHLVIDN RRIMNQASEF YLASSPPTGS FMDDSAMHIP PGLKPHPERM PPLIADSPKA RCPLDALNTT	SEQ ID NO: 6

KPKSLAVKKA KWHLGIRSQS KPYDIMAEVY
RAMKQLDFEW KVVNAYHLRV RRKNPVTGNY
VKMSLQLYLV DNRSYLLDFK SIDDEVLEQR
SGSSTPQRSC SAAGLHRPRS SLDSVTAESH
SLSGSLSGSL TGSMLPSVPP RLGSHTMDFF
EMCASLITTL AR

Human alpha-2 subunit	MAEKQKHDGR VKIGHYVLGD TLGVGTFGKV KIGEHQLTGH KVAVKILNRQ KIRSLDVVGK IKREIQNLKL FRHPHIIKLY QVISTPTDFF MVMEYVSGGE LFDYICKHGR VEEMEARRLF QQILSAVDYC HRHMVVHRDL KPENVLLDAH MNAKIADFGL SNMMSDGEFL RTSCGSPNYT APEVISGRLY AGPEVDIWSC GVILYALLCG TLPFDDEHVP TLFKKIRGGV FYIPEYLNRS VATLLMHMLQ VDPLKRATIK DIREHEWFKQ GLPSYLFPED PSYDANVIDD EAVKEVCEKF ECTESEVMNS LYSGDPQDQL AVAYHLIIDN RRIMNQASEF YLASSPPSGS FMDDSAMHIP PGLKPHPERM PPLIADSPKA RCPLDALNTT KPKSLAVKKA KWRQGIRSQS KPYDIMAEVY RAMKQLDFEW KVVNAYHLRV RRKNPVTGNY VKMSLQLYLV DNRSYLLDFK SIDDEVVEQR SGSSTPQRSC SAAGLHRPRS SFDSTTAESH SLSGSLTGSL TGSTLSSVSP RLGSHTMDFF EMCASLITTL AR	SEQ ID NO: 7

Human alpha-1 subunit	MRRLSSWRKM ATAEKQKHDG RVKIGHYILG DTLGVGTFGK VKVGKHELTG HKVAVKILNR QKIRSLDVVG KIRREIQNLK LFRHPHIIKL YQVISTPSDI FMVMEYVSGG ELFDYICKNG RLDEKESRRL FQQILSGVDY CHRHMVVHRD LKPENVLLDA HMNAKIADFG LSNMMSDGEF	SEQ ID NO: 8

LRTSCGSPNY AAPEVISGRL YAGPEVDIWS
SGVILYALLC GTLPFDDDHV PTLFKKICDG
IFYTPQYLNP SVISLLKHML QVDPMKRAAI
KDIREHEWFK QDLPKYLFPE DPSYSSTMID
DEALKEVCEK FECSEEEVLS CLYNRNHQDP
LAVAYHLIID NRRIMNEAKD FYLATSPPDS
FLDDHHLTRP HPERVPFLVA ETPRARHTLD
ELNPQKSKHQ GVRKAKWHLG IRSQSRPNDI
MAEVCRAIKQ LDYEWKVVNP YYLRVRRKNP
VTSTFSKMSL QLYQVDSRTY LLDFRSIDDE
ITEAKSGTAT PQRSGSISNY RSCQRSDSDA
EAQGKPSDVS LTSSVTSLDS SPVDVAPRPG
SHTIEFFEMC ANLIKILAQ

Yeast S. cerevisiae Beta subunit	MAGDNPENKD ASMLDVSDAA SNTTINGKHS ADSTNEASLA YTFSQMNVDN PNELEPQHPL RHKSSLIFND DDDDEIPPYS NHAENGSGET FDSDDDIDAS SSSSIDSNEG DIHDADMTGN TLQKMDYQPS QQPDSLQNQG FQQQQEQQQG TVEGKKGRAM MFPVDITWQQ GGNKVYVTGS FTGWRKMIGL VPVPGQPGLM HVKLQLPPGT HRFRFIVDNE LRFSDYLPTA TDQMGNFVNY MEVSAPPDWG NEPQQHLAEK KANHVDDSKL SKRPMSARSR IALEIEKEPD DMGDGYTRFH DETPAKPNLE YTQDIPAVFT DPNVMEQYYL TLDQQQNNHQ NMAWLTPPQL PPHLENVILN SYSNAQTDNT SGALPIPNHV ILNHLATSSI KHNTLCVASI VRYKQKYVTQ ILYTPLQ	SEQ ID NO: 9
Mouse Beta-1 subunit	MSNTSSERAA LERQAGHKTP RRDSSGGAKD GDRPKILMDS PEDADIFHSE EIKAPEKEEF LAWQHDLEAN DKAPAQARPT VFRWTGGGKE VYLSGSFNNW SKLPLTRSQN NFVAILDLPE GEHQYKFFVD GQWTHDPSEP IVTSQLGTVN	SEQ ID NO: 10

	NIIQVKKTDF EVFDALMVDS QKCSDVSELS SSPPGPYHQE PYMSKPEERF KAPPILPPHL LQVILNKDTG ISCDPALLPE PNHVMLNHLY ALSIKDGVMV LSATHRYKKK YVTTLLYKPI	
Human beta-1 subunit	MGNTSSERAA LERHGGHKTP RRDSSGGTKD GDRPKILMDS PEDADLFHSE EIKAPEKEEF LAWQHDLEVN DKAPAQARPT VFRWTGGGKE VYLSGSFNNW SKLPLTRSHN NFVAILDLPE GEHQYKFFVD GQWTHDPSEP IVTSQLGTVN NIIQVKKTDF EVFDALMVDS QKCSDVSELS SSPPGPYHQE PYVCKPEERF RAPPILPPHL LQVILNKDTG ISCDPALLPE PNHVMLNHLY ALSIKDGVMV LSATHRYKKK YVTTLLYKPI	SEQ ID NO: 11
Yeast S. cerevisiae Gamma subunit	MKPTQDSQEK VSIEQQLAVE SIRKFLNSKT SYDVLPVSYR LIVLDTSLLV KKSLNVLLQN SIVSAPLWDS KTSRFAGLLT TTDFINVIQY YFSNPDKFEL VDKLQLDGLK DIERALGVDQ LDTASIHPSR PLFEACLKML ESRSGRIPLI DQDEETHREI VVSVLTQYRI LKFVALNCRE THFLKIPIGD LNIITQDNMK SCQMTTPVID VIQMLTQGRV SSVPIIDENG YLINVYEAYD VLGLIKGGIY NDLSLSVGEA LMRRSDDFEG VYTCTKNDKL STIMDNIRKA RVHRFFVVDD VGRLVGVLTL SDILKYILLG SN	SEQ ID NO: 12
Human Gamma-1 subunit	METVISSDSS PAESNNSVYT SFMKSHRCYD LIPTSSKLVV FDTSLQVKKA FFALVTNGVR AAPLWDSKKQ SFVGMLTITD FINILHRYYK SALVQIYELE EHKIETWREV YLQDSFKPLV CISPNASLFD AVSSLIRNKI HRLPVIDPES GNTLYILTHK RILKFLKLFI TEFPKPEFMS KSLEELQIGT YANIAMVRTT TPVYVALGIF VQHRVSALPV VDEKGRVVDI YSKFDVINLA	SEQ ID NO: 13

AEKTYNNLDV SVTKALQHRS HYFEGVLKCY
LHETLETIIN RLVEAEVHRL VVVDENDVVK
GIVSLSDILQ ALVLTGGEKP

Pig Gamma-1 subunit	METVTSSDSS SAVENEHPQD TPESNNSVYT SFMKSHRCYD LIPTSSKLVV FDTSLQVKKA FFALVTNGVR AAPLWDSKKQ SFVGMLTITD FINILHRYYK SALVQIYELE EHKIETWREV YLQDSFKPLV CISPNASLFD AVSSLIRNKI HRLPVIDPES GNTLYILTHK RILKFLKLFI TEFPKPEFMS KSLEELQIGT YANIAMVRTT TPVYVALGIF VQHRVSALPV VDEKGRVVDI YSKFDVINLA AEKTYNNLDV SVTKALQHRS HYFEGVLKCY LHETLETIIN RLVEAEVHRL VVVDENDVVK GIVSLSDILQ ALVLTGGEKP	SEQ ID NO: 14

[0078] A three dimensional structure of any AMPK trimer core-ligand complex can be modeled using methods generally known in the art based on information obtained from analysis of an AMPK trimer core-ligand complex crystal, and from other AMPK trimer core-ligand complex structures which are derived from an AMPK trimer core-ligand complex crystal. The Examples section below discloses the production of an AMPK trimer core-ligand complex crystal, in particular an AMPK trimer core complexed with AMP, an AMPK trimer core complexed with ATP, or an AMPK trimer core complexed with 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (AICAR-phosphate, also called ZMP).

[0079] An aspect of the present teachings comprises using the three dimensional structure of one crystalline AMPK trimer core-ligand complex to derive the three dimensional structure of another AMPK trimer core-ligand complex. Therefore, a crystalline AMPK trimer core complexed with a ligand permits one of ordinary skill in the art to derive the three dimensional structure, and models thereof, of any AMPK trimer core-ligand complex. The derivation of the structure of any AMPK trimer core-ligand complex can be achieved even in the absence of having crystal structure data for such other AMPK trimer core-ligand complexes, and when the crystal structure of another AMPK trimer core-ligand complex is available, the modeling of the three dimensional structure of the new AMPK

trimer core-ligand complex can be refined using the knowledge already gained from the AMPK trimer core-ligand complex structure.

[0080] In some configurations of the present teachings, the absence of crystal structure data for other AMPK trimer core-ligand complexes, the three dimensional structures of other AMPK trimer core-ligand complex can be modeled, taking into account differences in the amino acid sequence of the other AMPK trimer core-ligand complex. Moreover, the present teachings allow for structure-based drug design of compounds which affect the activity of virtually any AMPK.

[0081] One aspect of the present teachings includes a three dimensional structure of an AMPK trimer core-ligand complex, in which the atomic coordinates of the AMPK trimer core-ligand complex are generated by a method comprising: (a) providing an AMPK trimer core complexed with a ligand in crystalline form; (b) generating an electron-density map of the crystalline AMPK trimer core complexed with the ligand; and (c) analyzing the electron-density map to produce the atomic coordinates. For example, the structure of human AMPK trimer core complexed with AMP or ATP are provided herein.

[0082] In some aspects, crystals of an AMPK trimer core-AMP complex can be prepared by rapidly thawing an AMPK trimer core, and diluting it 40 µl: 10 µl with 1 mM AMP pH 8.0 (5.5 mg/ml trimer core, 200 µM AMP). The trimer core-AMP mixture can be further mixed 1:1 with 7.2-6.2% PEG 6000, 10% Ethylene Glycol, 100mM Hepes pH 7.4 in a hanging drop. Similarly, crystals of an AMPK trimer core-ATP complex can be prepared by rapidly thawing an AMPK trimer core, and diluting it 40 µl: 10 µl with 5 mM ATP pH 8.0 (5.5 mg/ml trimer core, 1 mM ATP, 1 mM $MgCl_2$) The trimer core-ATP mixture can be further mixed 1:1 with 7.3-8.1% PEG 3350, 100mM Hepes pH 7.5 in a hanging drop. Crystals prepared by these methods can be used to generate initial datasets.

[0083] In various aspects of the present teachings, X-ray diffraction data for an AMPK trimer core in complex with a ligand can be collected on an individual crystal using methods well known to skilled artisans. In some configurations, multiwavelength anomalous diffraction with selenomethionine ("MAD," Hendrickson et al., EMBO J. 9: 1865-1672, 1990) can be used to elucidate the three dimensional structure of a crystal comprising

[0084] According to the present teachings, a three dimensional structure of one AMPK trimer core complexed with a ligand can be used to derive a model of the three dimensional structure of another AMPK trimer core-ligand complex (i.e., a structure to be

modeled). As used herein, a "structure" of a protein refers to the components and the manner of arrangement of the components to constitute the protein. As used herein, the term "model" refers to a representation in a tangible medium of the three dimensional structure of a protein, polypeptide or peptide. For example, a model can be a representation of the three dimensional structure in an electronic file, on a computer screen, on a piece of paper (i.e., on a two dimensional medium), and/or as a ball-and-stick figure. Physical three-dimensional models are tangible and include, but are not limited to, stick models and space-filling models. The phrase "imaging the model on a computer screen" refers to the ability to express (or represent) and manipulate the model on a computer screen using appropriate computer hardware and software technology known to those skilled in the art. Such technology is available from a variety of sources including, for example, Accelerys, Inc. (San Diego, Calif.). The phrase "providing a picture of the model" refers to the ability to generate a "hard copy" of the model. Hard copies include both motion and still pictures. Computer screen images and pictures of the model can be visualized in a number of formats including space-filling representations, α-carbon traces, ribbon diagrams and electron density maps.

[0085] Suitable target AMPK or AMPK trimer core-ligand complexes to model using methods of the present teachings include any AMPK or trimer core thereof, complexed with AMP, ATP, and/or 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (AICAR-phosphate, also called ZMP). In various aspects, a target AMPK trimer core-ligand complex structure that is substantially structurally related to a different AMPK trimer core-ligand complex can include a target AMPK trimer core-ligand complex structure having an amino acid sequence that is at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 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%, 99%, or 100% identical to an amino acid sequence of AMPK trimer core polypeptides, in particular amino acid sequences comprising, consisting essentially of, or consisting of a sequence set forth herein as SEQ ID NO: 1- SEQ ID NO: 3. In these configurations, a sequence alignment program such as BLAST (supra) can be used to aid in the analysis. In various aspects of the present teachings, target AMPK trimer core-ligand complex structures to model include proteins comprising amino acid sequences that are at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%,

63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 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%, 99%, or 100% identical to amino acid sequence SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3 when comparing suitable regions of the sequence, such as the amino acid sequence for a ligand or substrate binding site of any one of the amino acid sequences, when using an alignment program such as BLAST (supra) to align the amino acid sequences.

[0086] In various configurations of the present teachings, a structure can be modeled using techniques generally described by, for example, Sali, Current Opinions in Biotechnology, vol. 6, pp. 437-451, 1995, and algorithms can be implemented in program packages such as Insight II, available from Accelerys (San Diego, Calif.). Use of Insight II HOMOLOGY requires an alignment of an amino acid sequence of a known structure having a known three dimensional structure with an amino acid sequence of a target structure to be modeled. The alignment can be a pairwise alignment or a multiple sequence alignment including other related sequences (for example, using the method generally described by Rost, Meth. Enzymol., vol. 266, pp. 525-539, 1996) to improve accuracy. Structurally conserved regions can be identified by comparing related structural features, or by examining the degree of sequence identity between the known structure and the target structure. Certain coordinates for the target structure are assigned using known structures from the known structure. Coordinates for other regions of the target structure can be generated from fragments obtained from known structures such as those found in a resource such as the Protein Data Bank. Conformation of side chains of the target structure can be assigned with reference to what is sterically allowable and using a library of rotamers and their frequency of occurrence (as generally described in Ponder and Richards, J. Mol. Biol., vol. 193, pp. 775-791, 1987). The resulting model of the target structure, can be refined by molecular mechanics to ensure that the model is chemically and conformationally reasonable.

[0087] Another aspect of the present teachings is a method to derive a computer model of the three dimensional structure of a target ligand-complexed AMPK trimer core structure for which a crystal has been produced (referred to herein as a "crystallized target structure"). A suitable method to produce such a model includes the method comprising molecular replacement. Methods of molecular replacement are generally known by those of skill in the art and are performed in a software program including, for example, X-PLOR available from Accelerys (San Diego, Calif.). In various aspects, a crystallized target ligand-

complexed AMPK trimer core structure useful in a method of molecular replacement according to the present teachings has an amino acid sequence that is at least about 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 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%, 99%, or 100% identical to the amino acid sequence of the search structure (e.g., an AMPK), when the two amino acid sequences are compared using an alignment program such as BLAST (supra). A suitable search structure of the present teachings includes an AMPK trimer core-ligand complex having a three dimensional structure that substantially conforms with the atomic coordinates listed in Table 1 and/or Table 2.

[0088] Another aspect of the present teachings is a method for determining a three dimensional structure of a target AMPK trimer core-ligand complex. Such a method is useful for identifying structures that are related to the three dimensional structure of an AMPK trimer core-ligand complex based only on the three dimensional structure of the target structure. For example, the present method enables identification of structures that do not have high amino acid sequence identity with an AMPK protein but share three dimensional structure similarities of a ligand-complexed AMPK. In various aspects of the present teachings, a method to determine a three dimensional structure of a target AMPK trimer core-ligand complex structure can comprise: (a) providing an amino acid sequence of a target structure, wherein the three dimensional structure of the target structure is not known; (b) analyzing the pattern of folding of the amino acid sequence in a three dimensional conformation by fold recognition; and (c) comparing the pattern of folding of the target structure amino acid sequence with the three dimensional structure of an AMPK trimer core-ligand complex to determine the three dimensional structure of the target structure, wherein the three dimensional structure of the AMPK trimer core-ligand complex substantially conforms to the atomic coordinates represented in Table 1 and/or Table 2. For example, methods of fold recognition can include the methods generally described in Jones, Curr. Opinion Struc. Biol., vol. 7, pp. 377-387, 1997. Such folding can be analyzed based on hydrophobic and/or hydrophilic properties of a target structure.

[0089] One aspect of the present teachings includes a three dimensional computer image of the three dimensional structure of an AMPK trimer core-ligand complex. In one

aspect, a computer image is created to a structure which substantially conforms with the three dimensional coordinates listed in Table 1 and/or Table 2. A computer image of the present teachings can be produced using any suitable software program, including, but not limited to, PyMOL (supra). Suitable computer hardware useful for producing an image of the present teachings are known to those of skill in the art.

[0090] Another aspect of the present teachings relates to a computer-readable medium encoded with a set of three dimensional coordinates represented in Table 1 and/or Table 2, wherein, using a graphical display software program, the three dimensional coordinates create an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image. Yet another aspect of the present teachings relates to a computer-readable medium encoded with a set of three dimensional coordinates of a three dimensional structure which substantially conforms to the three dimensional coordinates represented in Table 1 and/or Table 2 wherein, using a graphical display software program, the set of three dimensional coordinates create an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.

[0091] The present teachings also include a three dimensional model of the three dimensional structure of a target structure, such a three dimensional model being produced by the method comprising: (a) providing an amino acid sequences of an AMPK comprised by an AMPK trimer core-ligand complex and amino acid sequences of a target AMPK trimer core-ligand complex structure; (b) identifying structurally conserved regions shared between the AMPK trimer core amino acid sequences and the amino acid sequence comprised by the target AMPK trimer core-ligand complex structure; (c) determining atomic coordinates for the target AMPK trimer core-ligand complex by assigning the structurally conserved regions of the target AMPK trimer core-ligand complex to a three dimensional structure using a three dimensional structure of the AMPK comprising an AMPK trimer core-ligand complex based on atomic coordinates that substantially conform to the atomic coordinates represented in Table 1 and/or Table 2 to derive a model of the three dimensional structure of the target AMPK trimer core-ligand complex. In one aspect, the model comprises a computer model.

[0092] Any isolated AMPK protein or trimer core thereof can be used with the methods of the present teachings. An isolated AMPK protein can be isolated from its natural milieu or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. To produce recombinant AMPK

protein, nucleic acid molecules encoding AMPK polypeptides can be inserted into any vector capable of delivering a nucleic acid molecule into a host cell. A nucleic acid molecule of the present teachings can encode any portion of an AMPK protein, in various aspects a full-length AMPK protein, and in various aspects polypeptides comprising a crystallizable trimer core. A suitable nucleic acid molecule to include in a recombinant vector, and particularly in a recombinant molecule, includes nucleic acid molecules encoding a polypeptides having amino acid sequences represented by SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

[0093] AMPK proteins or portions thereof of the present teachings can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, chromatofocusing and differential solubilization. In various aspects of the present teachings, an AMPK protein is purified in such a manner that the protein is purified sufficiently for formation of crystals useful for obtaining information related to the three dimensional structure of an AMPK trimer core-ligand complex. In some aspects, a composition of AMPK protein is about 70%, 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% pure.

[0094] Another aspect of the present teachings includes a composition comprising an AMPK trimer core-ligand complex in a crystalline form (i.e., AMPK trimer core-ligand complex crystals). In various aspects of the present teachings, a crystal ligand-complexed AMPK trimer core can be produced using the crystal formation method described in the Examples.

[0095] According to an aspect of the present teachings, a crystalline AMPK trimer core-ligand complex can be used to determine the ability of a compound of the present teachings to bind to an AMPK in a manner predicted by a structure based drug design method of the present teachings. In various aspects of the present teachings, an AMPK trimer core-ligand complex crystal is soaked in a solution containing a chemical compound of the present teachings. Binding of the chemical compound to the crystal is then determined by methods standard in the art such as those provided in the Examples section herein.

[0096] One aspect of the present teachings is a therapeutic composition. A therapeutic composition of the present teachings comprises one or more therapeutic compounds. The

therapeutic composition can be used for the treatment and/or prophylaxis of AMPK-associated diseases or conditions, such as those involved with disruption of cellular energy homeostasis. For example, compositions modulating AMPK activity can be used in the treatment of metabolic and/or cardiac disorders linked to AMPK activation/deactivation such as diabetes mellitus, obesity, atherosclerosis, cardiac ischemia, hypertrophic cardiomyopathy, Wolff-Parkinson-White syndrome re-entry arrhythmia; cancer; tumorigenesis, Peutz-Jeghers familial cancer syndrome, apoptosis, oxidative stress, and faulty mitochondrial biogenesis (see e.g., Dyck and Lopaschuk (2006) J Physiol 574(1), 95-112; Shen et al. (2006) Current Cardiology Reviews 2(2), 117-123(7); Shaw et al. (2004) PNAS 101(10), 3329-3335).

[0097] In one aspect, a therapeutic composition involving an AMPK is provided which promotes glucose uptake. For example, a therapeutic composition of the present teachings can stimulate activity of glucose transporters on a cell having an AMPK (e.g., eukaryotic cells).

[0098] In another aspect, a therapeutic composition involving an AMPK is provided which promotes chronic activation of AMPK as seen in a calorie restrictive diet (see Baur et al. (2006) Nature 444, 337 - 342). Such a composition can be used to treat obesity and/or high calorie diet-associated conditions such as accelerated aging, decreased lifespan, and/or cardiac and hepatic system damage.

[0099] In another aspect, a therapeutic composition involving an AMPK is provided which acts as an antifungal agent. For example, a therapeutic composition of the present teachings can inhibit infection and/or biofilm formation on synthetic materials, such as medical devices, caused by various microorganisms including, but not limited to, Candida spp. and Saccharomyces cerevisiae. AMPK/SNF1 is an essential activity in several human pathogens, including Candida albicans, and is linked to expression of fungal cell surface glycoproteins involved in adherence (see Kojic and Darouiche (2004) Clin Microbiol Rev 17, 255; Enloe et al. (2000) J Bacteriol 182, 5730; Kuchin et al. (2003) Biochem Soc Trans 31, 175; Vyas et al. (2003) Mol Cell Biol 23, 1341).

[0100] According to the present teachings, suitable therapeutic compounds of the present teachings include peptides or other organic molecules, and inorganic molecules. In various aspects, a therapeutic compound of the present teachings is not harmful (e.g., toxic) to a subject when such compound is administered to the subject. Suitable therapeutic compounds to design include peptides composed of "L" and/or "D" amino acids that are

configured as normal or retroinverso peptides, peptidomimetic compounds, small organic molecules, or homo- or hetero-polymers thereof, in linear or branched configurations.

[0101] Therapeutic compounds of the present teachings can be designed using structure-based drug design. In the present teachings, knowledge of the three dimensional structure of the nucleotide binding sites of an AMPK provide one of skill in the art the ability to design a therapeutic compound that 1) specifically binds to AMPKs, or to a selected subset of AMPKs, 2) is stable, and 3) results in stimulation or inhibition of a biological response such as glucose uptake activation.

[0102] Suitable structures and models useful for structure-based drug design are disclosed herein. Models of target structures to use in a method of structure-based drug design include models produced by any modeling method disclosed herein, such as, for example, molecular replacement and fold recognition related methods. In some aspects of the present teachings, structure based drug design can be applied to a structure of AMPK trimer cores in complex with AMP, ATP or mimetics or homologues thereof.

[0103] One aspect of the present teachings is a method for designing a drug which increases activity of an AMPK. In various configurations, the method comprises providing a three-dimensional structure of an AMPK trimer core-ligand complex comprising the AMPK and at least one ligand of the AMPK; and designing a chemical compound which is predicted to bind to the AMPK. The designing can comprise using physical models, such as, for example, ball-and-stick representations of atoms and bonds, or on a digital computer equipped with molecular modeling software. In some configurations, these methods can further include synthesizing the chemical compound, and evaluating the chemical compound for ability to interfere with an activity of the AMPK.

[0104] Suitable three dimensional structures of an AMPK trimer core-ligand complex and models to use with the present method are disclosed herein. According to the present teachings, designing a compound can include creating a new chemical compound or searching databases of libraries of known compounds (e.g., a compound listed in a computational screening database containing three dimensional structures of known compounds). Designing can also include simulating chemical compounds having substitute moieties at certain structural features. In some configurations, designing can include selecting a chemical compound based on a known function of the compound. In some configurations designing can comprise computational screening of one or more databases of compounds in

which three dimensional structures of the compounds are known. In these configurations, a candidate compound can be interacted virtually (e.g., docked, aligned, matched, interfaced) with the three dimensional structure of an AMPK trimer core-ligand complex by computer equipped with software such as, for example, the AutoDock software package, (The Scripps Research Institute, La Jolla, Calif.) or described by Humblet and Dunbar, Animal Reports in Medicinal Chemistry, vol. 28, pp. 275-283, 1993, M Venuti, ed., Academic Press. Methods for synthesizing candidate chemical compounds are known to those of skill in the art.

[0105] Various other methods of structure-based drug design are disclosed in references such as Maulik et al., 1997, Molecular Biotechnology: Therapeutic Applications and Strategies, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety. Maulik et al. disclose, for example, methods of directed design, in which the user directs the process of creating novel molecules from a fragment library of appropriately selected fragments; random design, in which the user uses a genetic or other algorithm to randomly mutate fragments and their combinations while simultaneously applying a selection criterion to evaluate the fitness of candidate ligands; and a grid-based approach in which the user calculates the interaction energy between three dimensional structures and small fragment probes, followed by linking together of favorable probe sites.

[0106] In one aspect, a chemical compound of the present teachings that binds to an AMPK trimer core-ligand complex can be a chemical compound having chemical and/or stereochemical complementarity with an AMPK trimer core, e.g., an AMPK ligand, such as, for example, AMP or ATP. In some configurations, a chemical compound that binds to an AMPK can associate with an affinity of at least about 10^{-6} M, at least about 10^{-7} M, or at least about 10^{-8} M.

[0107] Several sites of AMPKs can be targets for structure based drug design. These sites include, in non-limiting example residues which contact a ligand or substrate such as AMP or ATP (e.g., A196, A218, R290, S217, R141, R139, D308, S305, T191 (amino acids contacting AMP and/or ATP). Such sites may include several amino acids toward either the N- or C-terminus in addition to the specific listed amino acids.

[0108] In the present method of structure-based drug design, it is not necessary to align a candidate chemical compound (i.e., a chemical compound being analyzed in, for example, a computational screening method of the present teachings) to each residue in a target site. Suitable candidate chemical compounds can align to a subset of residues described

for a target site. In some configurations of the present teachings, a candidate chemical compound can have a conformation that promotes the formation of covalent or noncovalent binding between the target site and the candidate chemical compound. In certain aspects, a candidate chemical compound can bind to a surface adjacent to a target site to provide an additional site of interaction in a complex. For example, when designing an AMPK agonist, the antagonist can be designed to bind with sufficient affinity to the binding site or to substantially prohibit a ligand (i.e., a molecule that specifically binds to the target site) from binding to a target area. It will be appreciated by one of skill in the art that it is not necessary that the complementarity between a candidate chemical compound and a target site extend over all residues specified here.

[0109] In various aspects, the design of a chemical compound possessing stereochemical complementarity can be accomplished by means of techniques that optimize, chemically or geometrically, the "fit" between a chemical compound and a target site. Such techniques are disclosed by, for example, Sheridan and Venkataraghavan, Acc. Chem Res., vol. 20, p. 322, 1987; Goodford, J. Med. Chem., vol. 27, p. 557, 1984; Beddell, Chem. Soc. Reviews, vol. 279, 1985; Hol, Angew. Chem., vol. 25, p. 767, 1986; and Verlinde and Hol, Structure, vol. 2, p. 577, 1994, each of which are incorporated by this reference herein in their entirety.

[0110] Some aspects of the present teachings for structure-based drug design comprise methods of identifying a chemical compound that complements the shape of an AMPK or a structure that is related to an AMPK. Such method is referred to herein as a "geometric approach". In a geometric approach of the present teachings, the number of internal degrees of freedom (and the corresponding local minima in the molecular conformation space) can be reduced by considering only the geometric (hard-sphere) interactions of two rigid bodies, where one body (the active site) contains "pockets" or "grooves" that form binding sites for the second body (the complementing molecule, such as a ligand).

[0111] The geometric approach is described by Kuntz et al., J. Mol. Biol., vol. 161, p. 269, 1982, which is incorporated by this reference herein in its entirety. The algorithm for chemical compound design can be implemented using a software program such as AutoDock, available from The Scripps Research Institute (La Jolla, Calif.). One or more extant databases of crystallographic data (e.g., the Cambridge Structural Database System maintained by University Chemical Laboratory, Cambridge University, Lensfield Road,

Cambridge CB2 1EW, U.K. or the Protein Data Bank maintained by Rutgers University) can then be searched for chemical compounds that approximate the shape thus defined. Chemical compounds identified by the geometric approach can be modified to satisfy criteria associated with chemical complementarity, such as hydrogen bonding, ionic interactions or Van der Waals interactions.

[0112] In some aspects, a therapeutic composition of the present teachings can comprise one or more therapeutic compounds. A therapeutic composition of the present teachings can be used to treat disease in a subject such as, for example, a human in need of treatment, by administering such composition to the subject.

[0113] A therapeutic composition of the present teachings can also include an excipient, an adjuvant and/or carrier. Suitable excipients include compounds that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

[0114] In one aspect of the present teachings, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

[0115] Acceptable protocols to administer therapeutic compositions of the present teachings in an effective manner include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. Modes of administration can include, but are not

limited to, inhalation, subcutaneous, intradermal, intravenous, intranasal, oral, transdermal, intraocular and intramuscular routes.

[0116] Another aspect of the present teachings is a method for screening, generation, identification, and/or purification of compositions comprising compounds that interact with AMPK. Generally, compounds that interact with AMPK can be initially designed according to the approaches described above. Such designed compounds can then be screened for preferred characteristics, such as binding strength, stability, and/or stimulatory or inhibitory effects of AMPK activity. Also, compounds and/or compositions from libraries can be screened for the desired characteristics. Assays can be performed on living mammalian cells, which more closely approximate the effects of a particular serum level of drug in the body, on extracts prepared from the cultured cell lines, or on isolated AMPK, whether synthetically or biologically derived. Studies using extracts and isolated AMPK offer the possibility of a more rigorous determination of direct drug/enzyme interactions.

[0117] The screening methodology described herein can be, at least in part, based upon the observation that in an inactive conformation (e.g., ATP bound AMPK), the regulatory trimer does not associate with the kinase domain, but in an active conformation (e.g., for example, the AMP-bound), these domains bind together. Thus, activators will induce such association and antagonists will break this association. Screening for compounds (e.g., ligands) that induce association between these domains may identify agonists, and screening for compounds that break this association may identify antagonists.

[0118] Detection methods to screen for a compound's effect on association/dissociation of of/with the kinase domain encompass a wide variety of techniques as recognized in the art. The following is an exemplary summary of a generic protocol (see Bracha-Drori et al. (2005) Plant J. 40(3), 419-27, Erratum in (2005) Plant J. 42(5), 781). Each domain can be fluorescently labeled with a different fluorophore (e.g., GFP and CFP). Subsequently, the compound of interest can be contacted with the fluorescently labeled domains and the resulting fluorescence resonance energy transfer (FRET) evaluated. Generally, FRET signals will only be observed in the presence of agonists that induce association between the two domains. Similarly, antagonists can be found by screening in the presence of AMP. In this case, AMP can induce association seen as a FRET signal, and the addition of an antagonist that breaks this association will be observed as disappearance of the FRET signal. Alternatively, such an assay can be performed by bimolecular interaction

fluorescence complementation (BIFC), in which a different "half" of a fluorescent molecule (e.g., GFP) is grafted to each domain. When the domains come together, in the presence of an agonist, they will form a "whole" fluorescent molecule and fluorescence will be observed.

[0119] Having described the invention in detail, it will be apparent that modifications, variations, and equivalent embodiments are possible without departing the scope of the invention defined in the appended claims. Furthermore, it should be appreciated that all examples in the present disclosure are provided as non-limiting examples.

EXAMPLES

[0120] The following non-limiting examples are provided to further illustrate the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches the inventors have found function well in the practice of the invention, and thus can be considered to constitute examples of modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

[0121] The methods and compositions described herein utilize laboratory techniques well known to skilled artisans and can be found in laboratory manuals such as Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001; Spector, D. L. et al., Cells: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998; and Harlow, E., Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999. For pharmaceutical compositions, dosages and administration routes can be determined according to methods well-known in the art, for example, using methods provided by standard reference texts such as Remington: the Science and Practice of Pharmacy (Alfonso R. Gennaro ed. 19th ed. 1995); Hardman, J.G., et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, 1996; and Rowe, R.C., et al., Handbook of Pharmaceutical Excipients, Fourth Edition, Pharmaceutical Press, 2003.

EXAMPLE 1

[0122] This example illustrates generation of an AMPK trimer core

[0123] To define a crystallizable AMPK core for structural studies, full-length human α2β1γ1 AMPK trimers were produced by bacterial co-expression. Full-length trimers failed to yield crystals. However, analysis of limited digests with proteases trypsin and lys-C, followed by size exclusion chromatography, revealed a number of candidate fragments that retained heterotrimeric association. A recombinantly produced human α2β1γ1 trimer corresponding to one of these yielded crystals that diffracted x-rays to ~8 Å resolution. A corresponding trimer fragment from the AMPK gene of the yeast Schizosaccharomyces pombe was then produced, which yielded two distinct crystal forms that diffract x-rays to 2.6 Å (AMP complex) and 2.9 Å resolution (ATP complex). The trimer core consists of residues α 440-576, β 205-298, and the whole of the γ subunit 1-334 (Fig. 1). The structure omits two regions of the AMPK complex with previously-determined crystal structures: the N-terminal glycogen binding domain of the β subunit, and the N-terminal kinase domain of α. Significant sequence conservation among AMPKs from different species indicates that the structures presented here are representative of the class.

EXAMPLE 2

[0124] This example illustrates overall structure of the heterotrimeric adenylate sensor domain

[0125] Crystal structures were determined by selenomethionine MAD phasing for both AMP- and ATP-bound forms from crystals that each contained two trimers per asymmetric unit (Fig. 1 and Supp. Table I). Outside the localized nucleotide binding regions, no substantial structural changes attributable to bound nucleotide are observed among these four independent trimers. Each trimer is roughly triangular, with a wide base formed by the γ subunit, which associates with a tight αβ complex that forms the narrower apical domain (Fig 1A,B). The α subunit forms a compact mixed a/β domain (Fig. 5) that is topologically related to the kinase-associated domains (KAD) of MARK kinases. The β subunit lacks a hydrophobic core (Fig. 5B), and wraps wholly around the α KAD (Figs. 1 A,B), forming extensive hydrophobic contacts. These features suggest an obligate nature for αβ complexes.

[0126] The γ subunit forms an elliptical disk with an aqueous pore in the center. An adenine nucleotide is bound at the interface between CBS domains 3 and 4, positioning the phosphate groups in the pore. CBS domains 1 and 2 form the interface with the αβ complex (Figs. 1 A,B), and do not bind nucleotide. The γ interface to αβ is mediated primarily by interaction of a two-stranded β-sheet from the β-subunit, which hydrogen bonds

with the β1-strand of γ to form a 3-stranded sheet with β-strands contributed from both β and γ subunits (Fig. 1). In contrast to the aβ complex, very few hydrophobic interactions are formed between β and γ, with this interface formed mainly through hydrogen bonding and salt bridge interactions, suggesting the likelihood that the βγ interface is not obligatory. This is consistent with numerous reports demonstrating that γ can function independently of α and β in a variety of biological contexts (20, 29-31).

[0127] In each of the crystal forms described here, two heterotrimers associate to form a dimer-of-trimers with dimer contacts between α and γ subunits along the long edges of each triangle (Fig. 1C). Analytical ultracentrifugation analysis yields results consistent with a dimer-of-trimers, suggesting that this dimer-of-trimer configuration also obtains in solution. The dimer is pH dependent, dissociating above pH 7.5. The symmetry of the dimer is such that each of the adenylate molecules are bound on one face while the N-termini of the α subunits are situated on the opposite face (Fig.1D). This arrangement is geometrically consistent with the formation of a homodimer between kinase domains, as has been reported in prior crystallographic and biophysical studies of the AMPK α kinase domain from S. cerevisiae (32). This contrasts with results for the human heterotrimer, which does not appear to form higher order multimers (see below).

EXAMPLE 3

[0128] This example illustrates Adenylate binding

[0129] The γ subunit structures reported here provide the first views of cystathionine β-synthase (CBS) domains binding their regulatory ligands. A single molecule of either ATP or AMP binds at the same site, between β–strands 6 and 7 of CBS3 and β–strands 9 and 10 of CBS4 (Fig. 2). ATP and AMP bind in nearly identical conformations, utilizing the same set of ligating residues. In addition to these structural elements from CBS3 and CBS4, CBS2 also contributes critical interactions important for binding the nucleotide phosphates.

[0130] In both ATP and AMP-bound structures, a hydrophobic cleft formed from the side chains from CBS3 (I216 and P220) and CBS4 (I303 and F292) sandwich the adenine ring. The N2 ring nitrogen hydrogen bonds to backbone carbonyl groups of A196 and A218. The ribose moiety is bound in polar pocket in CBS4, in which the ribose 2' and 3' hydroxyls are hydrogen bonded to side chain atoms of T191, D308, and S305. In the AMP complex

structure (Fig. 1A), the terminal AMP α-phosphate forms salt bridges with the side chain of the conserved residue R290 in CBS3, and two residues in CBS2, R139 and R141. Excellent electron density is observed for all AMP atoms in the complex structure, and average B-values are 38.2, 43.2, and 49.3 $Å^2$ for base, sugar, and phosphate atoms, respectively. The ATP phosphates are ligated by an identical set of arginine side chains (Fig. 1B), however R290 ligates the terminal γ-phosphate, and R139 and R141 have poor density, and appear to adopt multiple conformations in coordinating the α- and β-phosphates (Fig. 1C).

[0131] The extra volume occupied by the larger phosphate structure of ATP is accommodated in two ways. First, the ATP triphosphate group adopts a compact helical structure that places the phosphate atoms near in space, with distances Pα-Pβ 2.71 Å, Pβ-Pγ 2.45 Å, and Pγ- Pα 5.07 Å, substantially lower than corresponding distances of 2.87 Å, 2.85 Å, and 5.48 Å found for the more representative ATP structure in an RNA ligase (PDB code 1XDN). The compact nature of the triphosphate is disfavored by electrostatic repulsion. As an apparent consequence, the average B-factors for bound ATP, 86.6, 100.8, and 128.5 $Å^2$ for base, sugar, and phosphate atoms, respectively are uniformly higher than for AMP. These structural findings are in agreement with the reported binding affinities of AMP (~100 μM) and ATP (~1 mM) for a protein encoding fragment the CBS3 and CBS4 domains of the human γ2 subunit (23). Cellular ATP levels are higher than AMP levels, often by an order of magnitude. Even when ATP levels fall, leading to AMPK activation, ATP concentrations generally remain higher than AMP. Thus, tighter binding to AMP is a functional requirement for AMPK. The structures presented here show how the enzyme achieves this requirement.

[0132] In the complex structures reported here, the CBS1/2 domain pair appears to lack or misposition structural elements critical for nucleotide binding (Figs. 2D,E). Three key features stand out: First, the distance between β-strands is smaller in the CBS1/2 interface, such that an analogously bound adenine base would clash with main chain atoms of CBS1. This difference appears to arise from the substitution of residues with smaller side chains than in CBS3/4, notably Pro144 in CBS1 which corresponds to Phe292 of CBS3; Second, the polar pocket of CBS3, which ligates the ribose hydroxyl groups, are absent from CBS1. Third, the phosphate groups of nucleotides bound in the CBS3/4 pocket are ligated by arginine side chains donated from CBS2. A similar mode of nucleotide binding in CBS1/2 would require the donation of positively charged side chains from CBS4 for phosphate binding. Although an arginine and a histidine residue are found at corresponding positions in CBS4, they are positioned so that they could not reach the phosphates of a bound nucleotide

in the CBS1/2 pocket (Fig. 2E). These three elements of structural divergence between CBS1/2 and CBS3/4 provide a rationale for the finding that, at least in the αβγ complex, AMPK binds only a single nucleotide.

[0133] Prior studies of AMP and ATP binding to recombinant bacterially-expressed fragments of human AMPK γ subunits suggested that each of two successive CBS domain pairs, CBS1/2 and CBS3/4, could bind a single nucleotide. However, in each structure reported here, determined in the presence of nucleotide concentrations well in excess of the reported dissociation constants for AMP and ATP, only a single nucleotide is bound at CBS3/4 interface. These seemingly contrary observations may be reconciled in two ways: First, the solution binding studies were performed with a recombinant human γ subunit which could function differently from the S. pombe protein. However, given the high similarity (Figs. 5, 6, and 7) between human and S. pombe γ subunits, this seems unlikely. A second possibility is that the γ subunit studied in isolation could have nucleotide binding properties that differ from the αβγ complex. This possibility is highlighted by the position in the complex of the β-flap, which caps the phosphate tunnel. The tunnel includes the region of CBS1/2 corresponding to the adenylate binding pocket of CBS3/4. Finally, if two nucleotides were to bind in the canonical orientation defined by the CBS3/4 structure the phosphates, the only region of difference between ATP and AMP, would be completely buried. It is difficult to understand how such a conformation could effect the differing functions of ATP and AMP by adopting such a structure. We conclude that, in the αβγ complex, AMPKs bind a single adenylate molecule.

[0134] The overall orientation of nucleotide binding, for both AMP and ATP, situates the nucleotide phosphates toward an internal cavity that we refer to as the "phosphate tunnel", which spans the breadth of the protein (Figs. 1A,B, 2, and 3). In both ATP- and AMP-bound forms, the surface-exposed nucleotide groups include parts of the adenine ring and the phosphate-distal face of the ribose moiety. These surface-exposed groups are identical in both the AMP- and ATP-bound structures, suggesting that the nucleotide-binding face of the AMPK regulatory trimer is unlikely to function as the site of activation modulated by adenylate binding. The primary differences between AMP- and ATP-bound forms of the heterotrimer lie within the phosphate tunnel, which appears to play a central role in kinase activation (see below).

EXAMPLE 4

[0135] This example illustrates structural mapping of function-disrupting mutants which highlight the phosphate tunnel

[0136] Prior studies have identified a number of mutations within the regulatory heterotrimer that lead to impaired function of AMPK, primarily in the γ subunit (1). These mutations include an insertion in helix E at position 91, and a point mutation at S247 (N488I in human γ2), which is found within the dimer-of-trimers interface region. However, the large majority of functionally important mutations, which include changes to residues (S. pombe numbering) R290, R141, V56, I55 T162, and R165, are all found lining the interior surface of the phosphate tunnel (Fig.3A). These residues, relative to the bound nucleotide phosphate groups, are positioned further toward the protein surface opposite to the nucleotide binding face. This face of the molecule likely constitutes the kinase domain (KD) interaction region.

[0137] The phosphate tunnel traverses the γ subunit, defining a large void that is capped on the KD-binding face by a polar loop from the β subunit (Fig. 3B). We refer to this loop, which includes residues 244-255 as the β-flap. The region of the β-flap that covers the phosphate tunnel includes only polar and charged residues, and makes no contacts to the hydrophobic core, suggesting the possibility for structural rearrangement. The β-flap appears to be highly mobile (average B-factors of 84.3 $Å^2$ in the four independent β subunits, as compared an overall average B-factor of 51.6 $Å^2$ for all protein atoms), and adopts slightly different conformations in the four independent copies of the structures presented here. The majority of γ-subunit mutations that affect AMPK activation are positioned within the phosphate tunnel, between the terminal phosphate of bound AXP and the β-flap. Since the difference between the inhibitory (ATP) and activating (AMP) ligands is in the number of phosphates placed within the tunnel, and mutants that affect kinase activation also lie within this tunnel, it appears likely that this represents the region of kinase domain interaction.

EXAMPLE 5

[0138] This example illustrates an ATP-dependent complex between the kinase domain and the adenylate sensor

[0139] Since nucleotide binding to the adenylate sensor regulates the kinase activity of the complex, we used solution biophysics measurements to test for interaction in trans between the regulatory heterotrimer and recombinantly-produced human α2 kinase

domain (residues 1-299). Analytical ultracentrifugation and surface plasmon resonance (SPR) experiments (Figs. 4A and 4B) performed with both human and S. pombe heterotrimeric sensor complexes, yielded consistent results. In the presence of 1mM AMP, the human kinase domain binds to the human adenylate sensor in a 1:1 complex with a dissociation constant on the order of 1-10 μM. However, in the presence of 1mM ATP, no binding is evident. Thus, the identity of the bound adenine nucleotide modulates the interaction between the regulatory and catalytic domains of AMPK.

[0140] Interactions between the S. pombe regulatory domain and the human kinase domain, which has about 93% sequence identity to the corresponding region from S. pombe AMPK, follows a similar pattern (Fig. 4B). In the presence of 1mM AMP, the regulatory and catalytic domains associate with K_D also on the order of 1-10 μM, however in 1mM ATP a complex is still observed, although with slightly reduced affinity. At higher concentrations of ATP (2 mM), however, complete dissociation of the complex is observed. This is in remarkable agreement with the differing ATP levels of yeast and human cells. ATP levels for human cells have been reported in the range of 10 μM for human prostrate cell lines (33) or 100 μM human β islet cells (34). For yeast cells, ATP levels are substantially higher, and have been reported in the range of 1.5mM (35). Thus, the adenylate sensors from S. pombe and human AMPK appear to be tuned to their respective cellular environments.

[0141] SPR and AUC experiments performed in the absence of nucleotide show that catalytic and adenylate sensor domains, for both the human and S. pombe enzymes, associate with affinities essentially equal to those measured in the presence of AMP (Fig. 4). This surprising finding suggests that the AMP-like complex, corresponding to the activated state, is the "default" for AMPKs, and the binding of ATP acts to dissociate this complex, leading to adoption of the inhibited state. This argues that AMPKs are not truly "activated" by AMP binding, but rather that apparent activation is achieved by the competitive displacement of ATP. Therefore, AMP-activated protein kinases would appear to be more correctly described as ATP-inhibited.

EXAMPLE 6

[0142] This example illustrates a model for AMPK regulation by adenylate energy charge.

[0143] We have shown that the heterotrimeric adenylate binding domain of S. pombe AMPK, which can serve as a model for the AMPK family, binds adenine nucleotides at a single site. Activating ligands such as AMP must therefore function by displacing the inhibitory ligand ATP. The positions of regulatory mutants in the adenylate sensor are clustered near the site of the bound nucleotide phosphates, the only region of structural difference between activating and inhibitory ligands. Solution binding experiments show that, for both human and yeast enzymes, the interaction of regulatory and catalytic domains is modulated by the identity of the bound nucleotide: In the activated (AMP-bound) state, regulatory and catalytic domains form 1:1 complexes with micromolar affinity, yet in the inhibited (ATP-bound) state, no binding is observed between the two AMPK subdomains. These data suggest that binding between the regulatory and catalytic domains defines the molecular event responsible for enzyme activation. The observation of binding in the absence of a nucleotide ligand indicates that inhibition by ATP ligation, rather than proper activation by AMP, represents the primary means of nucleotide-mediated enzyme regulation.

[0144] Based on these data, it is possible to propose a simple mechanism for AMPK regulation (Fig. 4C) in which either AMP or ATP binds from the nucleotide binding face of the regulatory trimer, so as to position their differing phosphate moieties within the traversing tunnel of the γ subunit. The likely site of kinase domain interaction is demarcated by the position of the mutant cluster between the bound phosphates and the opposite face of the regulatory trimer which constitutes the putative KD-interacting region.

[0145] This mechanism requires that the catalytic kinase domain exist in an inhibited state when ATP is bound. This inhibited state, at least for the yeast enzymes, can correspond to the active site-to-active site dimer found in the crystal structure of the kinase domain from S. cerevisiae AMPK (32, 36). This dimer configuration, which sterically precludes the interaction of substrates, can indeed correspond to an auto-inhibited state. Notably, the related calmodulin (37, 38) and MARK (39) kinase families are also thought to be regulated by auto-inhibition through kinase domain homodimerization. In the S. pombe AMPK regulatory dimer-of-heterotrimers, both putative KD-interacting faces lie on the same side of a large protein platform. Binding of the kinase domains to the two KD-interacting sites of the regulatory heterotrimers, as occurs in the absence of bound ATP, would be geometrically inconsistent with the auto-inhibited kinase domain homodimer. Thus, when ATP is displaced, association between KD and regulatory domains would break the homodimer contacts between kinase domains to provide relief of auto-inhibition (Fig. 4C).

[0146] The structures and biophysical interaction analyses presented here provide a basis for understanding the regulation of AMPKs by adenylate energy charge. The overall regulatory mechanism, whereby ATP dissociates the active heterotrimer/kinase domain complex, appears to be conserved from yeast to man. However, there is currently no evidence that human AMPKs form dimers suggesting the possibility that other mechanisms may be involved in the inhibition of kinase activity. Nonetheless, the data presented here suggest that the activation mechanism will be the same. The primary role of ATP rather than AMP levels in regulation of AMPK may provide important advantages for metabolic control. The expenditure of ATP in most metabolic processes yields ADP, with the exception of DNA replication in which AMP is generated. Thus, ATP levels should yield a better and more immediate measure of metabolic activity. In recent years, AMPK has received considerable attention for its potential as a drug target for metabolic diseases, including diabetes and obesity (40). The new understanding of AMPK activation and the high-resolution structures presented here should provide important new tools in the rational development of therapeutics.

[0147] All publications, patents, patent applications, and other references cited in this application are incorporated herein by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application or other reference was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Citation of a reference herein shall not be construed as an admission that such is prior art to the present invention.

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CLAIMS

What is claimed is:

1. A method for designing a drug which enhances activity of an adenosine monophosphate-activated kinase (AMPK), the method comprising:

(a) providing on a digital computer a three-dimensional structure of an AMPK trimer core-ligand complex comprising the trimer core of an AMPK and an AMPK ligand; and

(b) using software comprised by the digital computer to design a chemical compound which is predicted to bind to the AMPK trimer core.

2. A method in accordance with claim 1, wherein the AMPK trimer core comprises polypeptides consisting of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

3. A method in accordance with claim 1, wherein the AMPK trimer core comprises polypeptides consisting essentially of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

4. A method in accordance with claim 1, wherein the AMPK trimer core comprises polypeptides having amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

5. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 70% sequence identity with a trimer core polypeptide of a human AMPK.

6. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 80% sequence identity with a trimer core polypeptide of a human AMPK.

7. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 90% sequence identity with a trimer core polypeptide of a human AMPK.

8. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 95% sequence identity with a trimer core polypeptide of a human AMPK.

9. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 70% sequence identity with a trimer core polypeptide of a Schizosaccharomyces pombe AMPK.

10. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 80% sequence identity with a trimer core polypeptide of a Schizosaccharomyces pombe AMPK.

11. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 90% sequence identity with a trimer core polypeptide of a Schizosaccharomyces pombe AMPK.

12. A method in accordance with claim 1, wherein the AMPK trimer core consists essentially of polypeptides each having at least about 95% sequence identity with a trimer core polypeptide of a Schizosaccharomyces pombe AMPK.

13. A method in accordance with claim 1, further comprising:

(c) synthesizing the chemical compound; and

(d) evaluating the chemical compound for an ability to enhance an activity of the AMPK.

14. A method in accordance with claim 1, wherein the adenosine phosphate is selected from the group consisting of adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate.

15. A method in accordance with claim 1, wherein the adenosine phosphate is selected from the group consisting of adenosine monophosphate (AMP) and adenosine triphosphate.

16. A method in accordance with claim 1, wherein the ligand is an AMP analogue or mimetic.

17. A method in accordance with claim 16, wherein the AMP mimetic is 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (ZMP).

18. A method according to claim 1, wherein the chemical compound is designed by computational interaction with reference to a three dimensional site of the structure of the AMPK trimer core-ligand complex, wherein the three dimensional site comprises one or more amino acids, wherein each amino acid is selected from the group consisting of A196, A218, R290, S217, R141, R139, D308, S305, and T191.

19. A method for designing a drug which inhibits activity of an adenosine monophosphate-activated kinase (AMPK), the method comprising:

(a) providing on a digital computer a three-dimensional structure of an AMPK trimer core-ligand complex comprising the trimer core of an AMPK and an AMPK ligand; and

(b) using software comprised by the digital computer to design a chemical compound which is predicted to bind to the AMPK trimer core.

20. A method in accordance with claim 19, wherein the AMPK trimer core consists of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

21. A method in accordance with claim 19, wherein the AMPK trimer core consists essentially of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

22. A method in accordance with claim 19, wherein the AMPK trimer core comprises amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

23. A method in accordance with claim 19, further comprising:

(c) synthesizing the chemical compound; and

(d) evaluating the chemical compound for an ability to enhance an activity of the AMPK.

24. A method in accordance with claim 19, wherein the adenosine phosphate is selected from the group consisting of adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate.

25. A method in accordance with claim 19, wherein the adenosine phosphate is selected from the group consisting of adenosine monophosphate (AMP) and adenosine triphosphate.

26. A method in accordance with claim 19, wherein the ligand is an AMP analogue or mimetic.

27. A method in accordance with claim 26, wherein the AMP mimetic is 5-

aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5`-monophosphate (ZMP).

28. A method according to claim 19, wherein the chemical compound is designed by computational interaction with reference to a three dimensional site of the structure of the AMPK trimer core-ligand complex, wherein the three dimensional site comprises one or more AMPK amino acids, wherein each AMPK amino acid is selected from the group consisting of A196, A218, R290, S217, R141, R139, D308, S305, and T191.

29. A method for generating a model of a three dimensional structure of a target AMPK or a trimer core thereof, the method comprising:

(a) providing amino acid sequences of a reference AMPK and an amino acid sequence of a target AMPK, wherein atomic coordinates for an AMPK trimer core-ligand complex of the reference AMPK are known;

(b) identifying conserved regions shared between the reference AMPK and the target AMPK; and

(c) assigning atomic coordinates from the conserved regions to the target AMPK.

30. A method in accordance with claim 29, wherein the trimer core of the reference AMPK consists of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

31. A method in accordance with claim 29, wherein the trimer core of the reference AMPK consists essentially of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

32. A method in accordance with claim 29, wherein the trimer core of the reference AMPK comprises amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

33. A method in accordance with claim 29, wherein the target AMPK consists of amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

34. A method in accordance with claim 29, wherein the target AMPK consists essentially of amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

35. A method in accordance with claim 29, wherein the trimer core of the target AMPK comprises amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

36. A method in accordance with claim 29, wherein the ligand is AMP and the reference AMPK trimer core-ligand complex has a three dimensional structure described by atomic coordinates that substantially conform to atomic coordinates set forth in Table 1 .

37. A method in accordance with claim 29, wherein the ligand is ATP and the reference AMPK trimer core-ligand complex has a three dimensional structure described by atomic coordinates that substantially conform to atomic coordinates set forth in Table 2.

38. A method for determining a three dimensional structure of a target AMPK trimer core-ligand complex, the method comprising:

(a) providing amino acid sequences of the target AMPK or a trimer core thereof;

(b) predicting the pattern of folding of the amino acid sequence in a three dimensional conformation using a fold recognition algorithm; and

(c) comparing the pattern of folding of the target structure amino acid sequence with the three dimensional structure of a reference AMPK trimer core-ligand complex.

39. A method in accordance with claim 38, wherein the trimer core of the reference AMPK consists of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

40. A method in accordance with claim 38, wherein the trimer core of the reference AMPK consists essentially of amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

41. A method in accordance with claim 38, wherein the trimer core of the reference AMPK comprises amino acid sequences as set forth in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3.

42. A method in accordance with claim 38, wherein the reference AMPK consists of amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

43. A method in accordance with claim 38, wherein the reference AMPK consists essentially of amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

44. A method in accordance with claim 38, wherein the trimer core of the reference AMPK comprises amino acid sequences as set forth in SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5.

45. A method in accordance with claim 38, wherein the reference AMPK trimer core-ligand complex comprises a three dimensional structure described by atomic coordinates that substantially conform to atomic coordinates of an AMPK trimer core-ligand complex set forth in table 1 or table 2 .

46. A method for increasing adenosine monophosphate-activated protein kinase

(AMPK) activity, the method comprising:

selecting an AMPK agonist by performing a structure based drug design using a three-dimensional structure determined for a crystal comprising a trimer core of an AMPK and an AMPK ligand; and

contacting a sample comprising AMPK with the agonist.

47. A method in accordance with claim 46, wherein the AMPK is comprised by a human patient in need of treatment with the AMPK agonist.

48. A method in accordance with claim 46, wherein the AMPK ligand is a nucleotide.

49. A method in accordance with claim 48, wherein the nucleotide is selected from the group consisting of AMP, ADP and ATP.

50. A method in accordance with claim 48, wherein the nucleotide is selected from the group consisting of AMP and ATP.

51. A method in accordance with claim 46, wherein the AMPK trimer core comprised by the crystal is a yeast AMPK trimer core.

52. A method in accordance with claim 51, wherein the yeast AMPK trimer core is a Schizosaccharomyces pombe AMPK trimer core.

53. A method in accordance with claim 46, wherein the selecting comprises:

a) performing a structure-based drug design using a three-dimensional structure determined for a crystal of an AMPK trimer core-ligand complex to identify a candidate agonist;

b) contacting the candidate agonist with an AMPK; and

c) detecting an increase of at least one activity of the AMPK.

54. A method in accordance with claim 46, wherein the AMPK is a human AMPK.

55. A method for inhibiting adenosine monophosphate-activated kinase (AMPK) activity, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

contacting a sample or subject comprising an AMPK with the agonist.

56. A method in accordance with claim 55, wherein the subject is a human subject in need of treatment.

57. A method in accordance with claim 55, wherein the selecting comprises:

a) performing a structure-based drug design using a three-dimensional structure determined for a crystal of an AMPK trimer core-ligand complex to identify a candidate inhibitor;

b) contacting the candidate agonist with an AMPK; and

c) detecting enhancement of at least one activity of the AMPK.

58. A method in accordance with claim 55, wherein the AMPK is a human AMPK.

59. A method in accordance with claim 55, wherein the AMPK trimer core is a trimer core of a yeast AMPK.

60. A method in accordance with claim 59, wherein the yeast is a Schizosacharromyces pombe.

61. A crystal comprising an AMPK trimer core and an AMPK ligand.

62. A crystal in accordance with claim 61, wherein the ligand is a nucleotide or a nucleotide mimetic.

63. A crystal in accordance with claim 61, wherein the ligand is a nucleotide selected from the group consisting of AMP, ADP, and ATP.

64. A crystal in accordance with claim 61, wherein the ligand is a nucleotide selected from the group consisting of AMP and ATP.

65. A crystal in accordance with claim 62, wherein the nucleotide mimetic is 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (ZMP).

66. A crystal in accordance with claim 61, wherein the ligand is AMP.

67. A crystal in accordance with claim 66, wherein the crystal comprises space group $P2_12_12_1$ so as to form a unit cell of dimensions a~73.5 Å, b~97.4 Å, c~168.9 Å, or wherein the crystal comprises space group $P2_1$ so as to form a unit cell of dimensions a~46.2 Å, b~39.9 Å, c~66.5 Å, and β~110.7°.

68. A crystal in accordance with claim 66, wherein the AMP contacts the AMPK trimer core at one or more amino acids selected from the group consisting of A196, A218, R290, S217, R141, R139, D308, S305, and T191.

69. A crystal in accordance with claim 61, wherein the ligand is ATP.

70. A crystal in accordance with claim 69, wherein the crystal comprises space group $P2_12_12_1$ so as to form a unit cell of dimensions a~73.5 Å, b~97.4 Å, c~168.9 Å, or wherein the crystal comprises space group $P2_1$ so as to form a unit cell of dimensions a~46.2 Å, b~39.9 Å, c~66.5 Å, and β~110.7°.

71. A crystal in accordance with claim 69, wherein the ATP contacts the AMPK trimer core at one or more amino acids selected from the group consisting of A196, A218, R290, S217, R141, R139, D308, S305, and T191.

72. A crystal comprising AMPK trimer core complexed with an AMPK ligand, wherein the crystal is sufficiently ordered to determine atomic coordinates of the complex by X-ray diffraction to a resolution of about 2.6 Å.

73. A crystal in accordance with claim 72, wherein the AMPK ligand is AMP.

74. A crystal comprising AMPK trimer core complexed with an AMPK ligand, wherein the crystal is sufficiently ordered to determine atomic coordinates of the complex by X-ray diffraction to a resolution of about 2.9 Å.

75. A crystal in accordance with claim 74, wherein the AMPK ligand is ATP.

76. A therapeutic compound which increases AMPK activity, wherein the compound is selected by a) performing a structure based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; b) contacting a sample comprising AMPK with the compound, and c) detecting enhancement of at least one activity of the AMPK.

77. A therapeutic compound which inhibits AMPK activity, wherein the compound is selected by a) performing a structure based drug design using a three-

dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; b) contacting a sample comprising AMPK with the compound, and c) detecting inhibition of at least one activity of the AMPK.

78. A three dimensional computer image of the three dimensional structure of an AMPK trimer core-ligand complex, wherein the structure substantially conforms with the three dimensional coordinates listed in Table 1 or Table 2.

79. A computer-readable medium encoded with a set of three dimensional coordinates set forth in Table 1 or Table 2, wherein using a graphical display software program, the three dimensional coordinates of Table 1 or Table 2 comprise data for an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.

80. A computer-readable medium encoded with a set of three dimensional coordinates of a three dimensional structure which substantially conforms to the three dimensional coordinates represented in Table 1 or Table 2, wherein using a graphical display software program, the set of three dimensional coordinates comprise data for an electronic file that can be visualized on a computer capable of representing said electronic file as a three dimensional image.

80. A method of forming a crystal comprising an AMPK trimer core and an AMPK ligand, the method comprising:

forming a composition comprising the AMPK trimer core, the ligand, and water; and

adding a solution comprising polyethylene glycol, ethylene glycol, and a buffer.

81. A method in accordance with claim 80, wherein the AMPK trimer core is a yeast AMPK trimer core and the AMPK ligand is a nucleotide.

82. A method in accordance with claim 81, wherein the nucleotide is selected

from the group consisting of AMP, ADP and ATP.

83. A method in accordance with claim 81, wherein the nucleotide is selected from the group consisting of AMP and ATP.

84. A method in accordance with claim 80, wherein the AMPK trimer core is a yeast AMPK trimer core and the AMPK ligand is a nucleotide mimetic.

85. A method in accordance with claim 84, wherein the nucleotide mimetic is 5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside 5'-monophosphate (ZMP).

86. A method in accordance with claim 80, wherein the AMPK trimer core is a yeast AMPK trimer core.

87. A method in accordance with claim 86, wherein the yeast is Schizosaccharomyces pombe.

88. A method in accordance with claim 80, wherein the AMPK trimer core is a human AMPK trimer core.

89. A method in accordance with claim 80, wherein the AMPK trimer core is a humanized AMPK trimer core.

90. A method in accordance with claim 80, further comprising digesting an AMPK with at least one protease.

91. A method in accordance with claim 90, wherein the at least one protease is selected from the group consisting of trypsin and lys C.

92. A method for elevating AMPK activity in a subject for the treatment of a disease, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

93. A method in accordance with claim 92, wherein the disease is diabetes.

94. A method in accordance with claim 93, wherein the diabetes is type 2 diabetes.

95. A method for stimulating glucose uptake in a cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

contacting the cell or tissue with an effective amount of the agonist.

96. A method in accordance with claim 95, wherein the cell or tissue is a muscle cell or tissue.

97. A method in accordance with claim 96, wherein the muscle cell or tissue is a skeletal muscle cell or tissue.

98. A method of achieving whole-body glycemic control, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a

three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

99. A method of decreasing glucose production in a liver cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

100. A method of decreasing lipid synthesis in a liver cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

101. A method of increasing lipid oxidation in a liver cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

102. A method of decreasing lipolysis in a cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a

three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

103. A method in accordance with claim 102, wherein the cell or tissue is an adipose cell or tissue.

104. A method of decreasing lipogenesis in a cell or tissue, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

105. A method in accordance with claim 104, wherein the cell or tissue is an adipose cell or tissue.

106. A method of decreasing circulating lipid levels, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

107. A method in accordance with claim 106, wherein the cell or tissue is an adipose cell or tissue.

108. A method of decreasing ectopic fat deposition, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a

three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

109. A method in accordance with claim 108, wherein the cell or tissue is an adipose cell or tissue.

110. A method of decreasing insulin secretion by the pancreas, the method comprising:

selecting an agonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the agonist to a subject in need thereof.

111. A method in accordance with any one of claims 95-110, wherein the selecting comprises:

a) identifying a candidate agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal of a complex comprising an AMPK trimer core and an AMPK ligand;

b) contacting the candidate agonist with an AMPK; and

c) detecting enhancement of at least one activity of the AMPK.

112. A method in accordance with any one of claims 95-110, wherein the AMPK is a human AMPK.

113. A method in accordance with any one of claims 95-110, wherein the AMPK trimer core is a yeast AMPK trimer core.

114. A method of promoting longevity, the method comprising administering to a subject an agonist of AMPK.

115. A method of promoting longevity in accordance with claim 114, wherein the method further comprises selecting an AMPK agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising a trimer core of an AMPK and an AMPK ligand.

116. A method for decreasing AMPK activity in a subject for the treatment of a disease, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

117. A method for inhibiting glucose uptake in a cell or tissue, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

contacting the cell or tissue with an effective amount of the antagonist.

118. A method in accordance with claim 117, wherein the cell or tissue is a muscle cell or tissue.

119. A method in accordance with claim 118, wherein the muscle cell or tissue is a skeletal muscle cell or tissue.

120. A method of increasing glucose production in a liver cell or tissue, the method

comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

121. A method of increasing lipid synthesis in a liver cell or tissue, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

122. A method of decreasing lipid oxidation in a liver cell or tissue, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

123. A method of increasing lipolysis in a cell or tissue, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

124. A method in accordance with claim 121, wherein the cell or tissue is an

adipose cell or tissue.

125. A method of increasing lipogenesis in a cell or tissue, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

126. A method in accordance with claim 124, wherein the cell or tissue is an adipose cell or tissue.

127. A method of increasing circulating lipid levels, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

128. A method in accordance with claim 126, wherein the cell or tissue is an adipose cell or tissue.

129. A method of increasing ectopic fat deposition, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

130. A method in accordance with claim 128, wherein the cell or tissue is an

adipose cell or tissue.

131. A method of increasing insulin secretion by the pancreas, the method comprising:

selecting an antagonist of AMPK by performing a structure-based drug design using a three-dimensional structure determined for a crystal comprising an AMPK trimer core and an AMPK ligand; and

administering a therapeutically effective amount of the antagonist to a subject in need thereof.

132. A method in accordance with any one of claims 116-131 wherein the selecting comprises:

a) identifying a candidate agonist by performing a structure-based drug design using a three-dimensional structure determined for a crystal of a complex comprising an AMPK trimer core and an AMPK ligand;

b) contacting the candidate antagonist with an AMPK; and

c) detecting inhibition of at least one activity of the AMPK.

133. A method in accordance with any one of claims 116-132, wherein the AMPK is a human AMPK.

134. A method in accordance with any one of claims 116-121, wherein the AMPK trimer core is a yeast AMPK trimer core.

135. A high throughput screening method for identification of AMPK agonists comprising the steps of:

providing a candidate compound;

providing an AMPK trimer core and an AMPK kinase domain;

contacting the candidate compound, the AMPK trimer core, and the AMPK kinase domain;

detecting association of the AMPK trimer core and the AMPK kinase domain;

correlating association of the AMPK trimer core and an AMPK kinase domain with agonist activity of the candidate compound.

136. A high throughput screening method for identification of AMPK antagonists comprising the steps of:

providing a candidate compound;

providing an AMPK trimer core, an AMPK kinase domain, and an AMPK ligand;

contacting the candidate compound, the AMPK trimer core, the AMPK kinase domain, and the AMPK ligand;

detecting dissociation of the AMPK trimer core and the AMPK kinase domain;

correlating dissociation of the AMPK trimer core and the AMPK kinase domain with antagonist activity of the candidate compound.

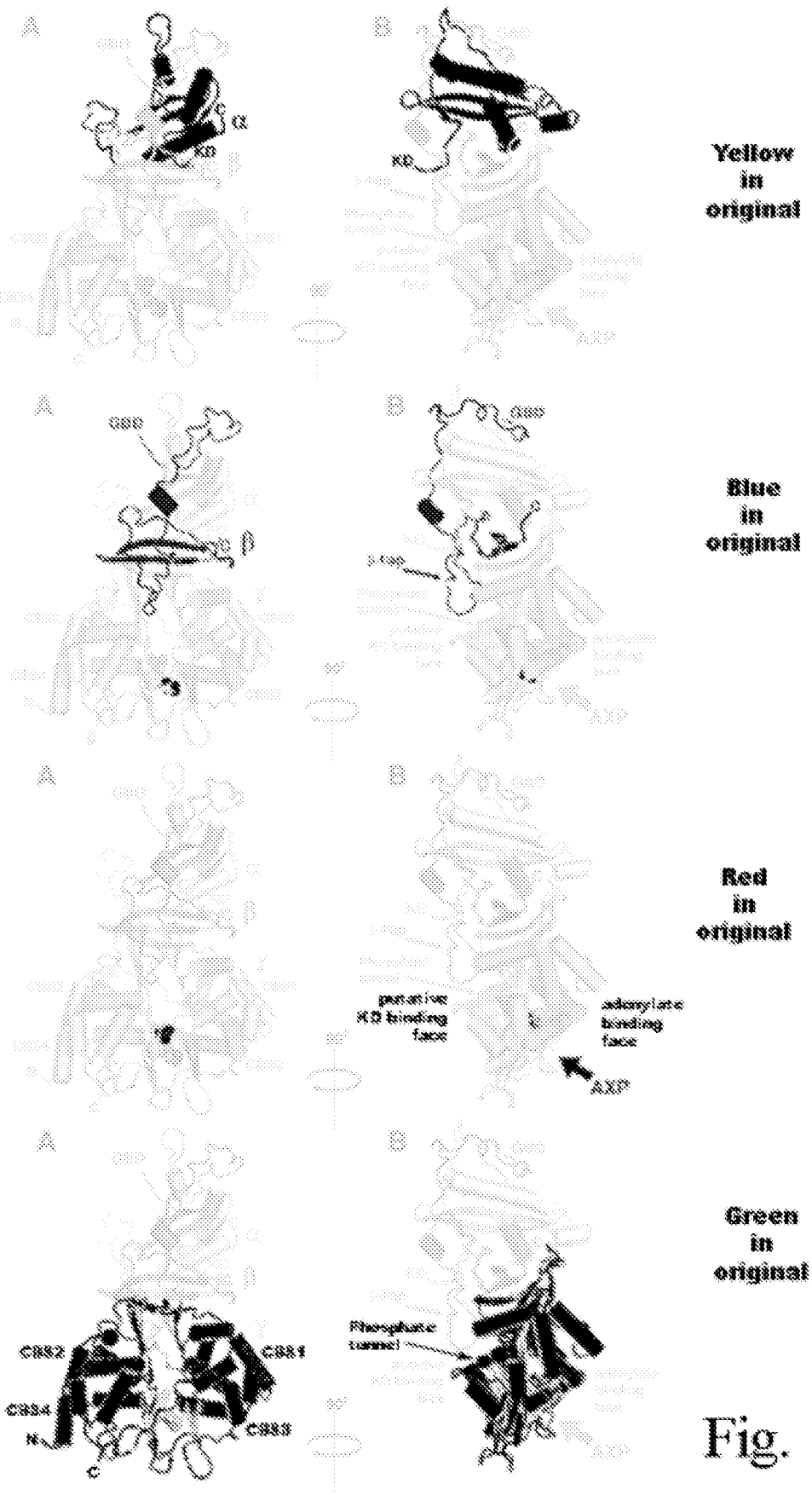

A
α
KD
B
KD
Yellow in original
A
CBD
β
B
CBD
Blue in original
A
B
putative KD binding face
adenylate binding face
ATP
Red in original
A
CBS2
CBS1
CBS4
CBS3
N
C
B
Phosphate tunnel
Green in original

Fig. 1A-B


C
Yellow in original
D
C
Blue in original
D
C
Green in original
D

Fig. 1C-D

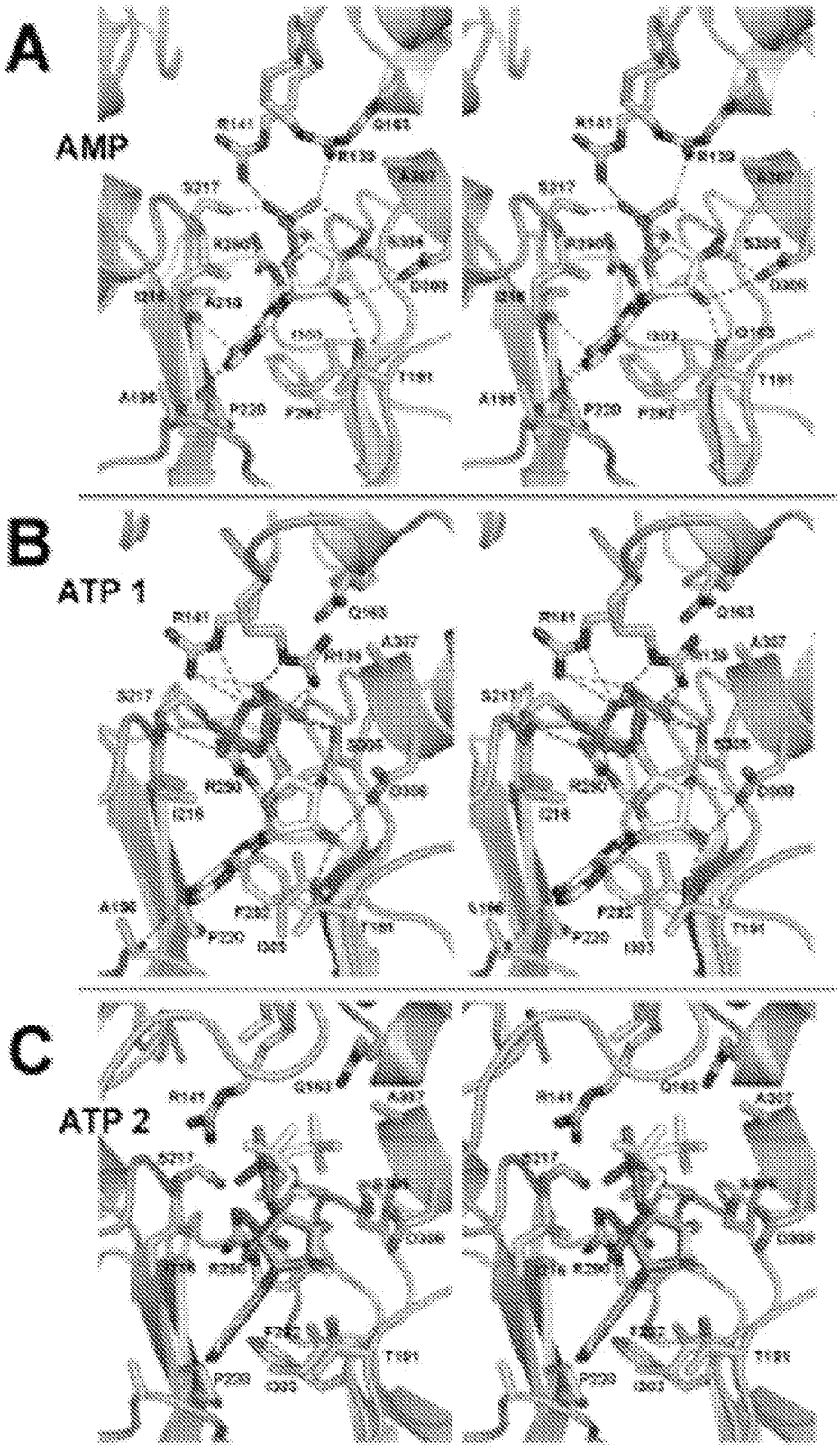
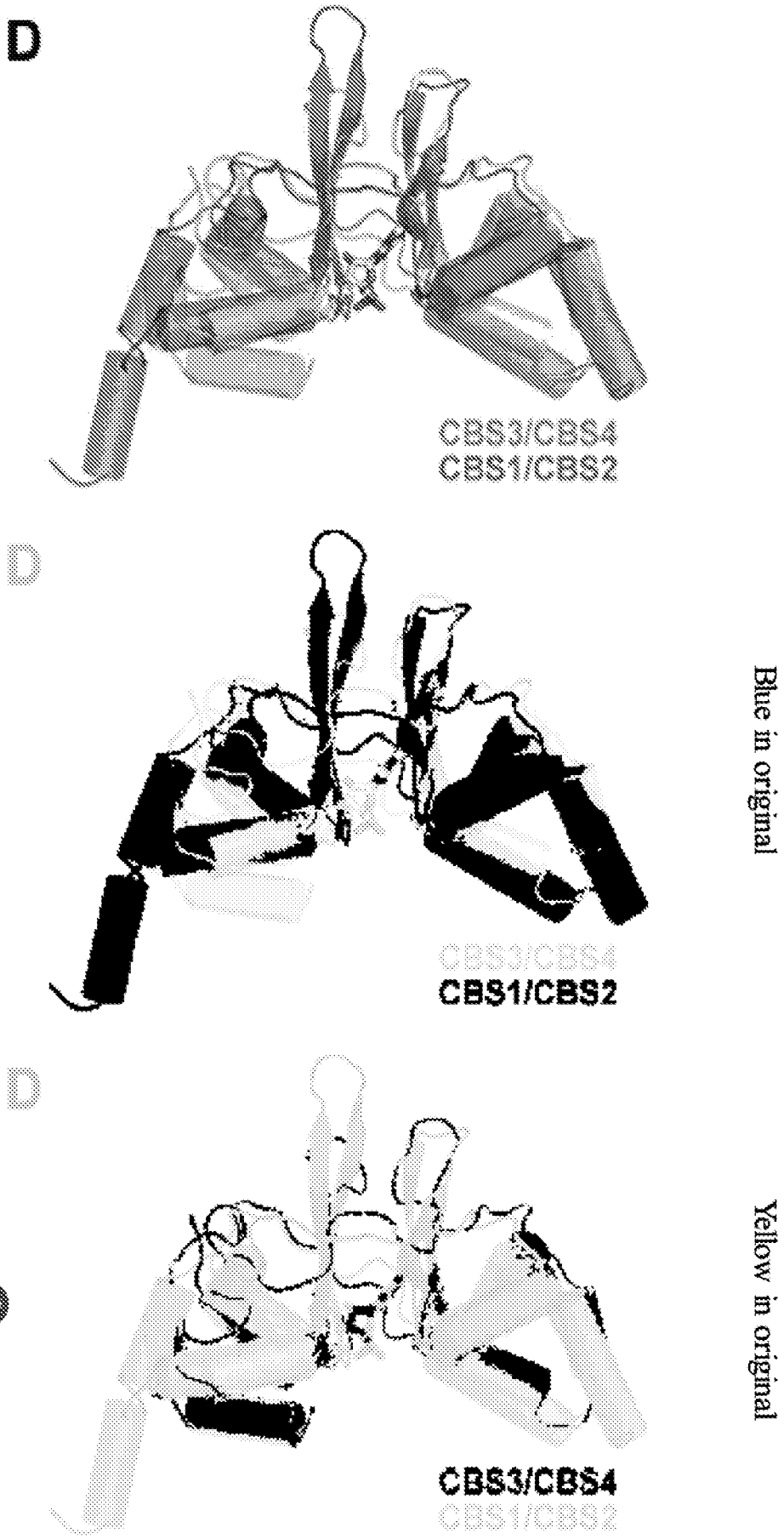


Fig. 2A-C



Blue in original

Yellow in original

Fig. 2D

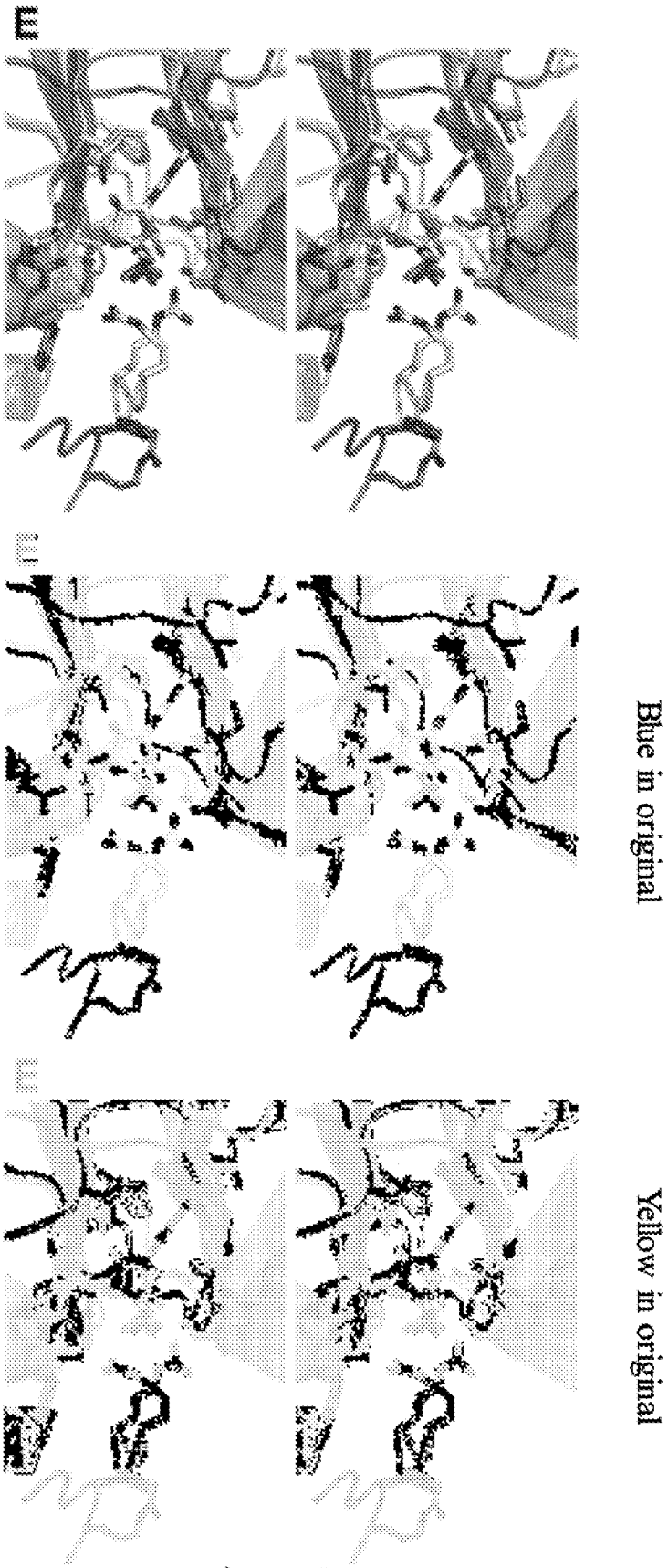
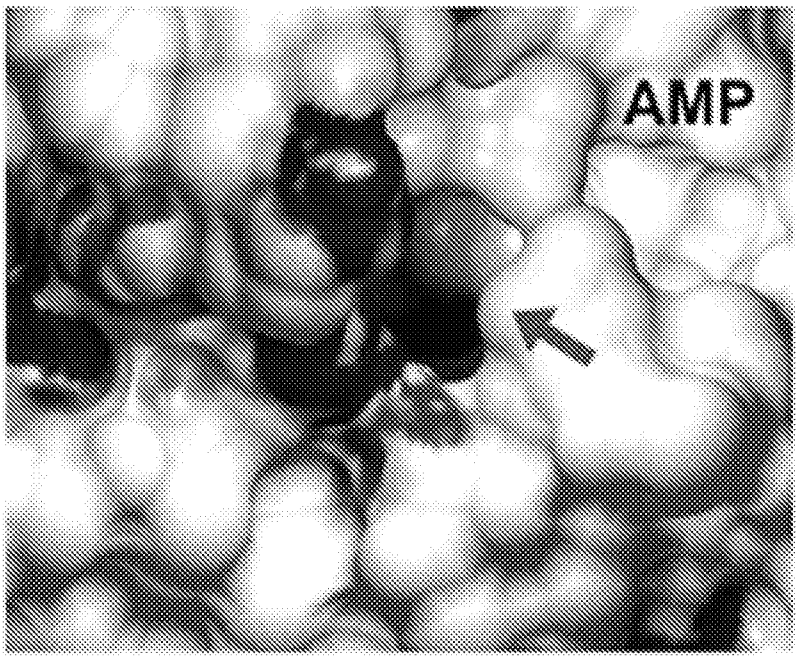


Fig. 2E

F



G

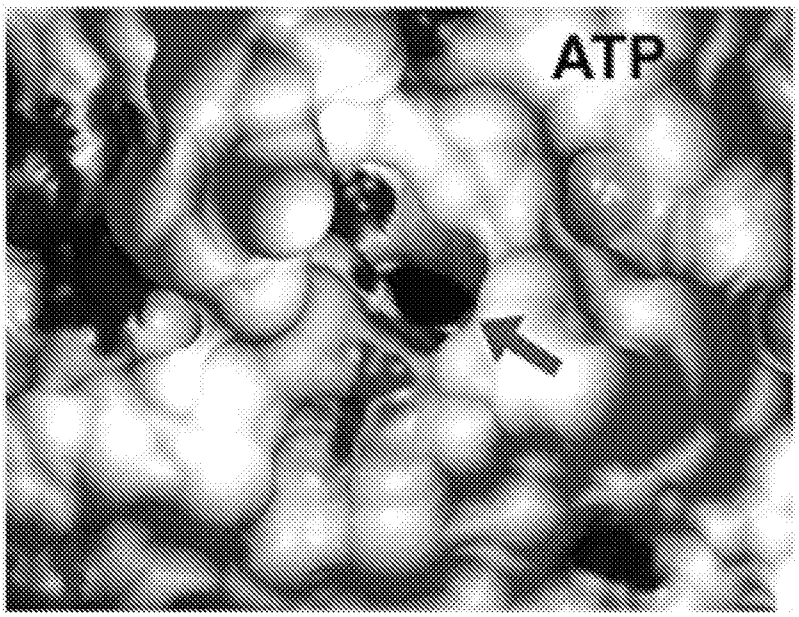


Fig. 2F-G

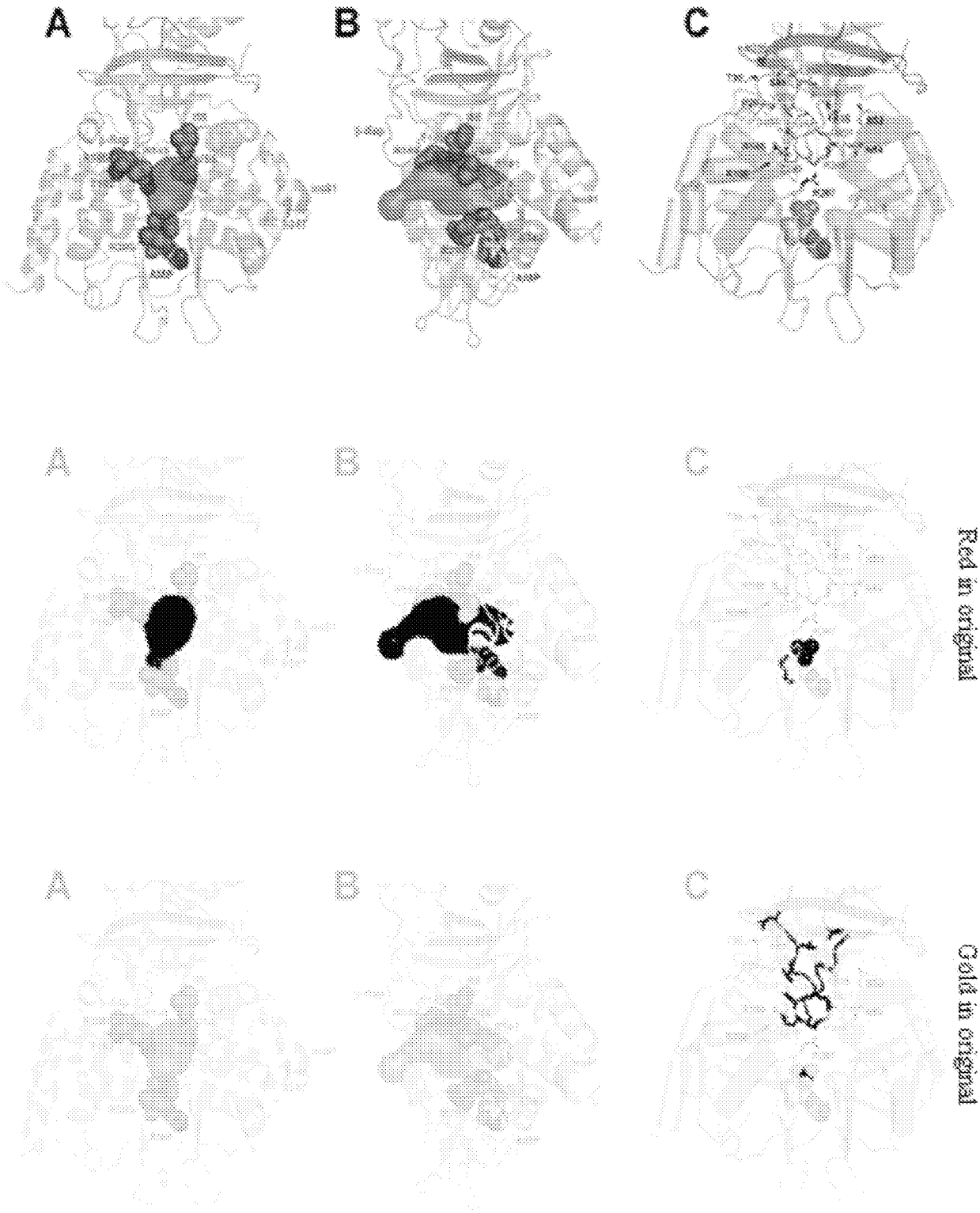


Fig. 3

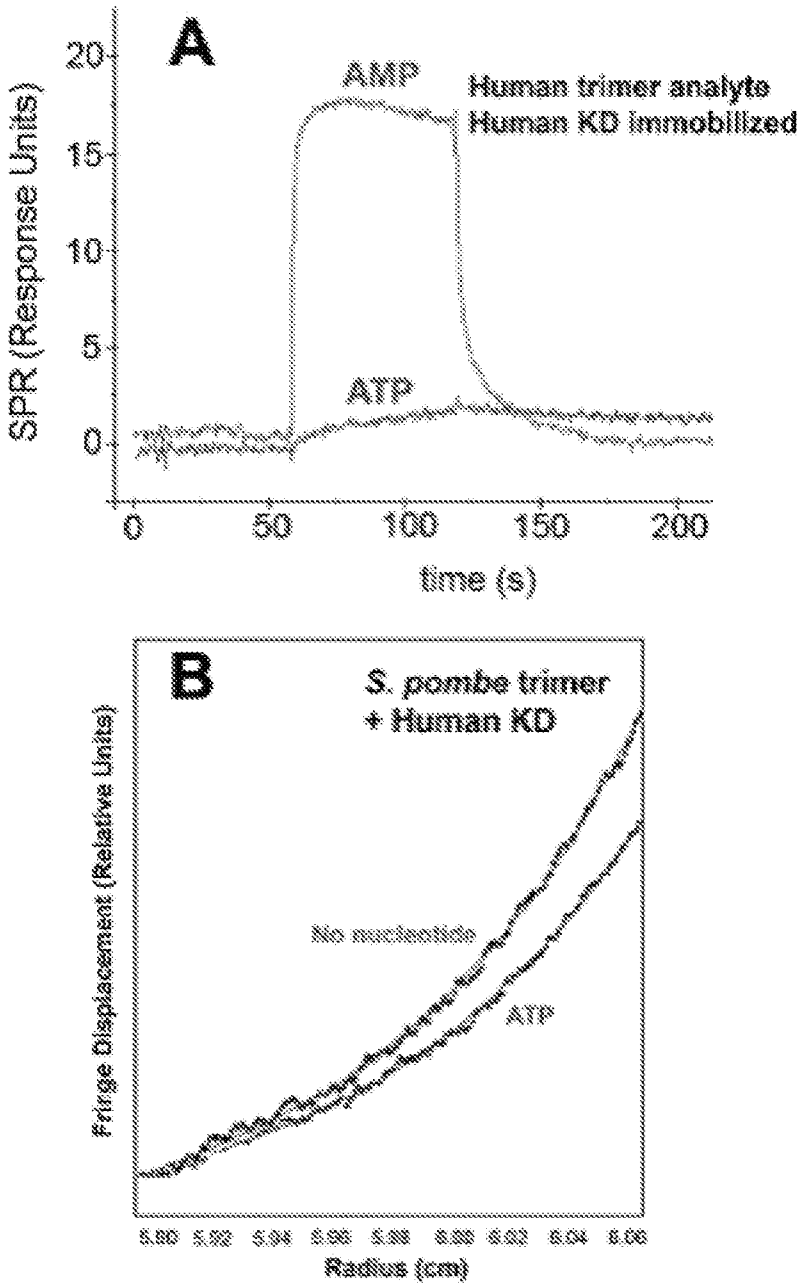

A
AMP
Human trimer analyte
Human KD immobilized
ATP
SPR (Response Units)
20
15
10
5
0
0
50
100
150
200
time (s)
B
S. pombe trimer
+ Human KD
No nucleotide
ATP
Fringe Displacement (Relative Units)
Radius (cm)

Fig. 4A-B

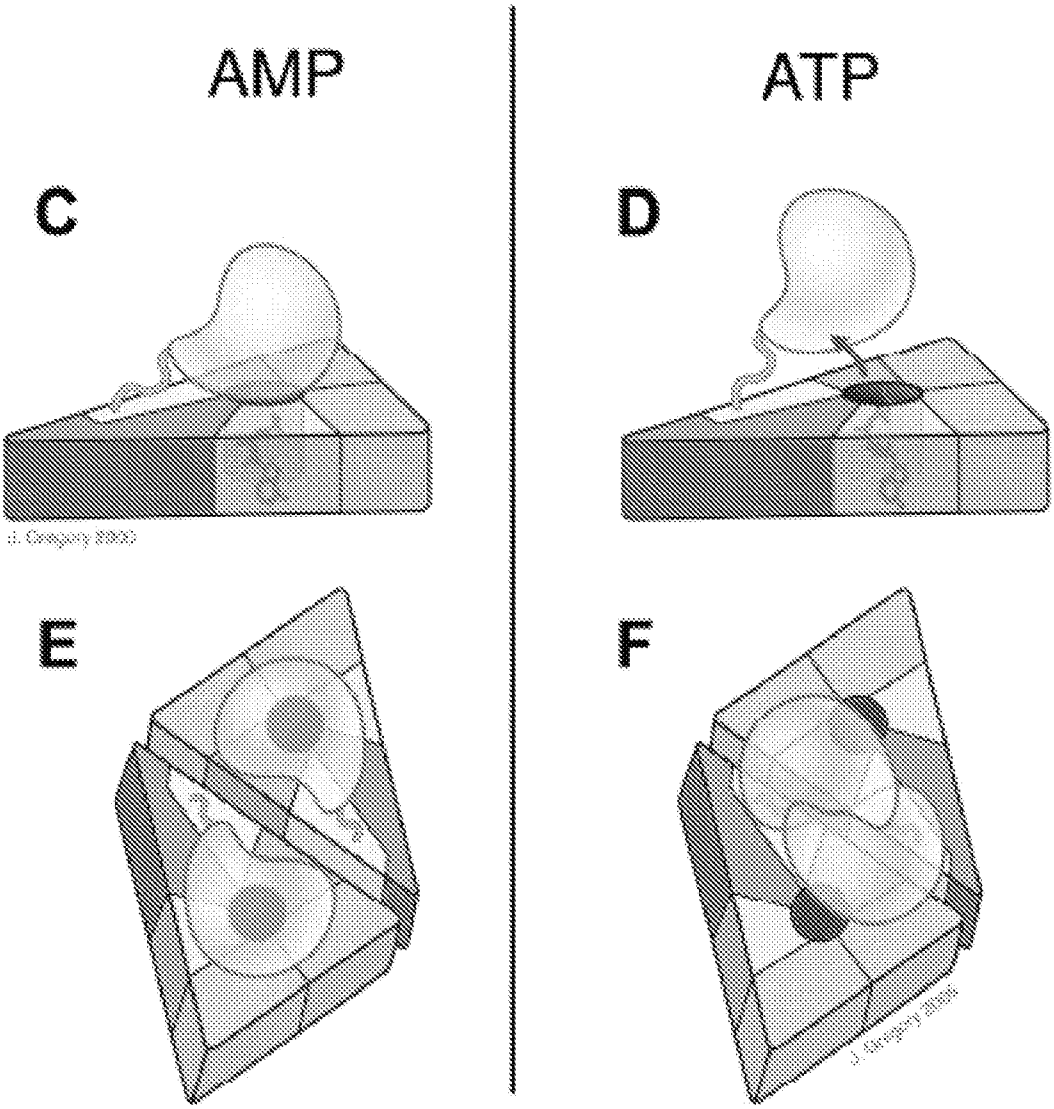


Fig. 4C-F

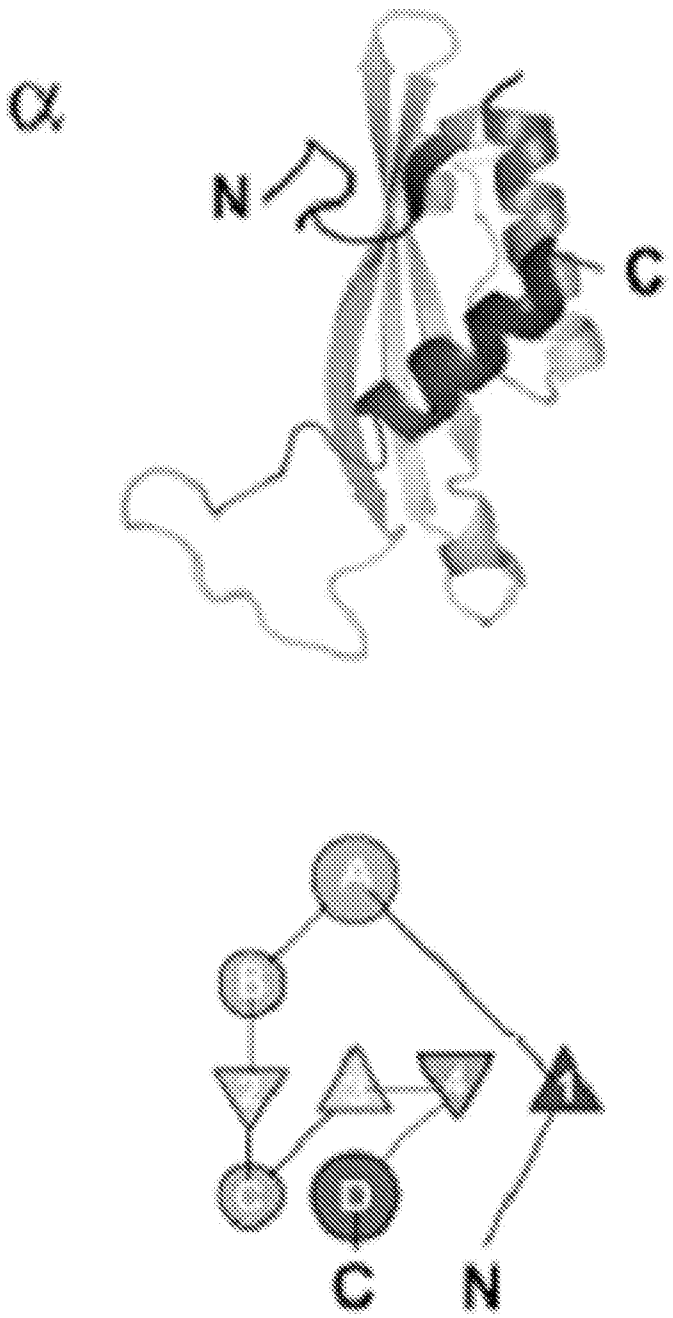


Fig. 5A

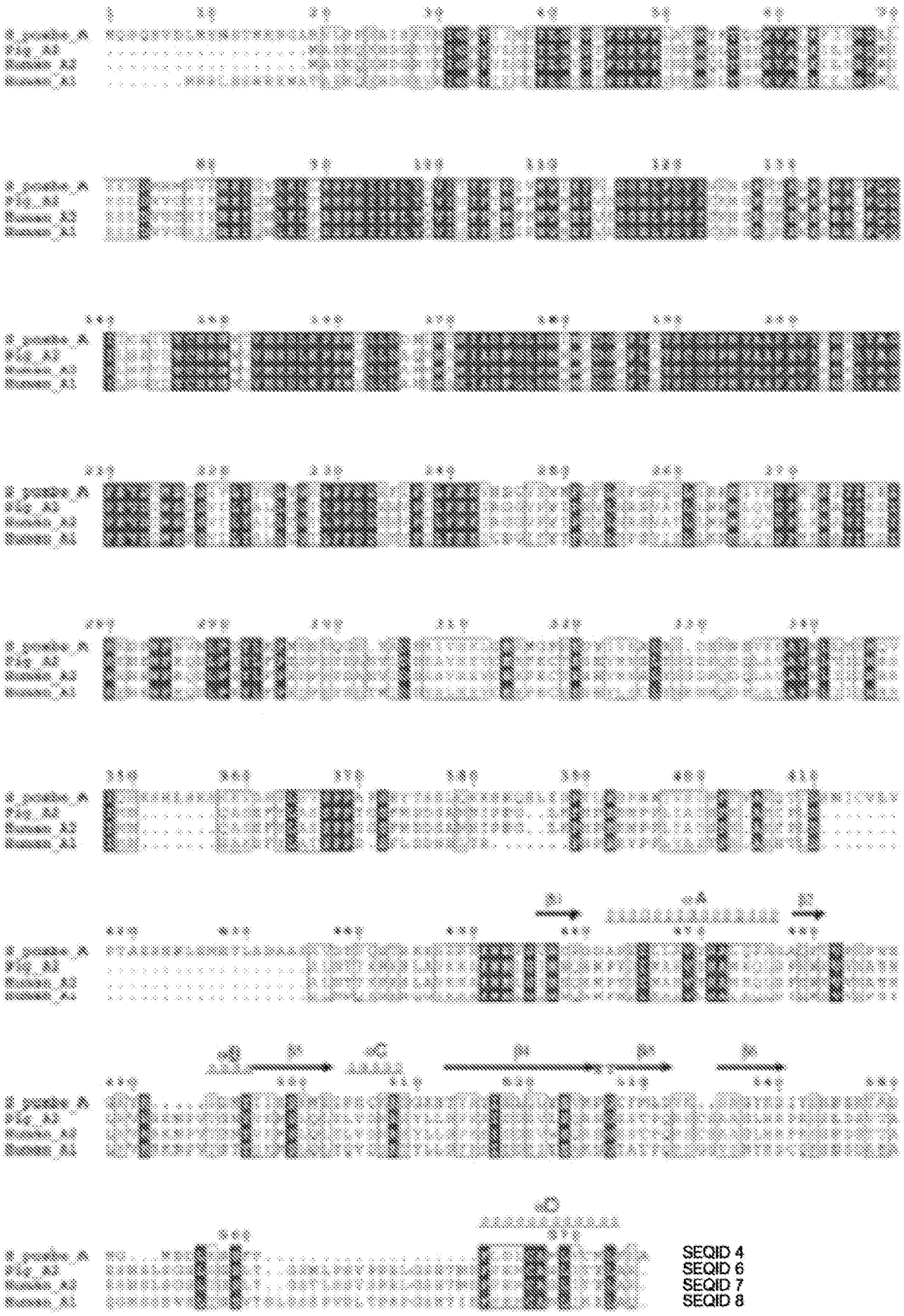


Fig. 5B

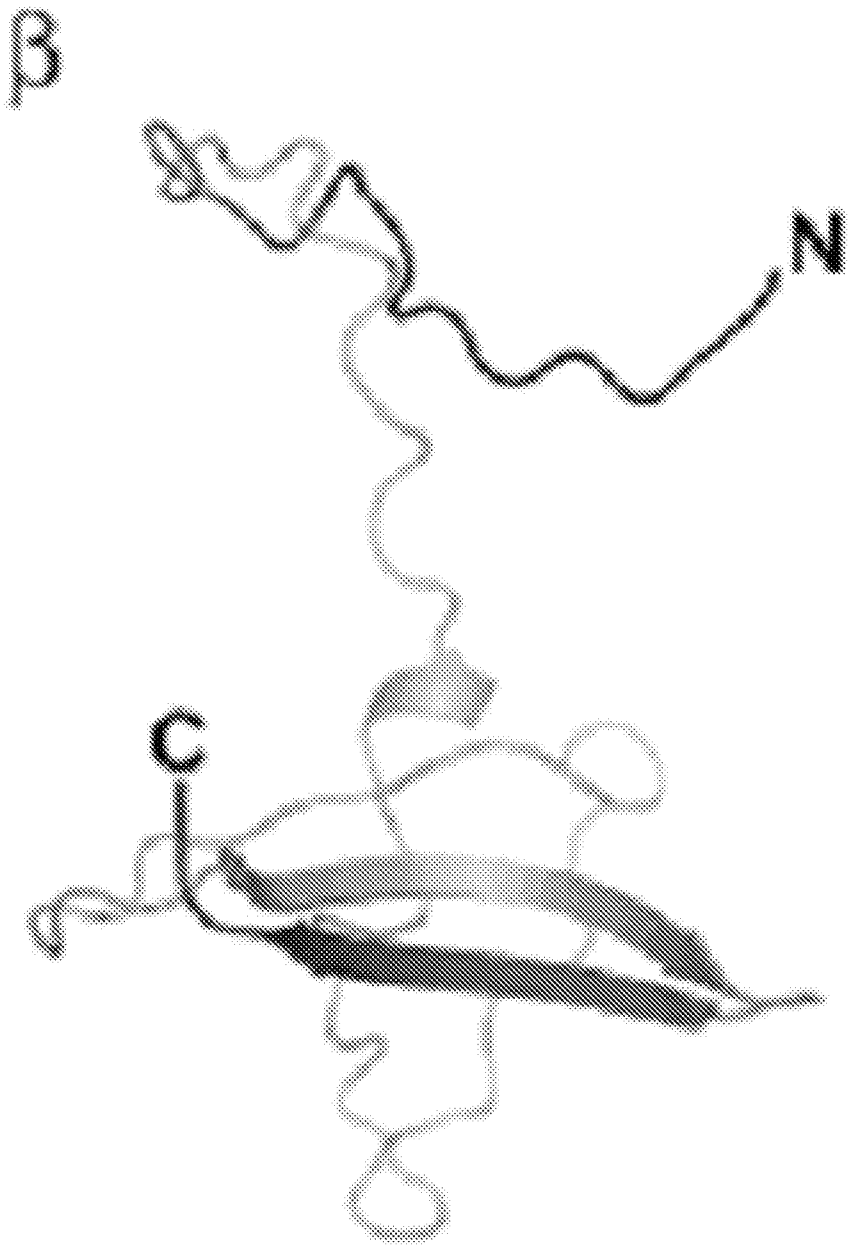


Fig. 6A

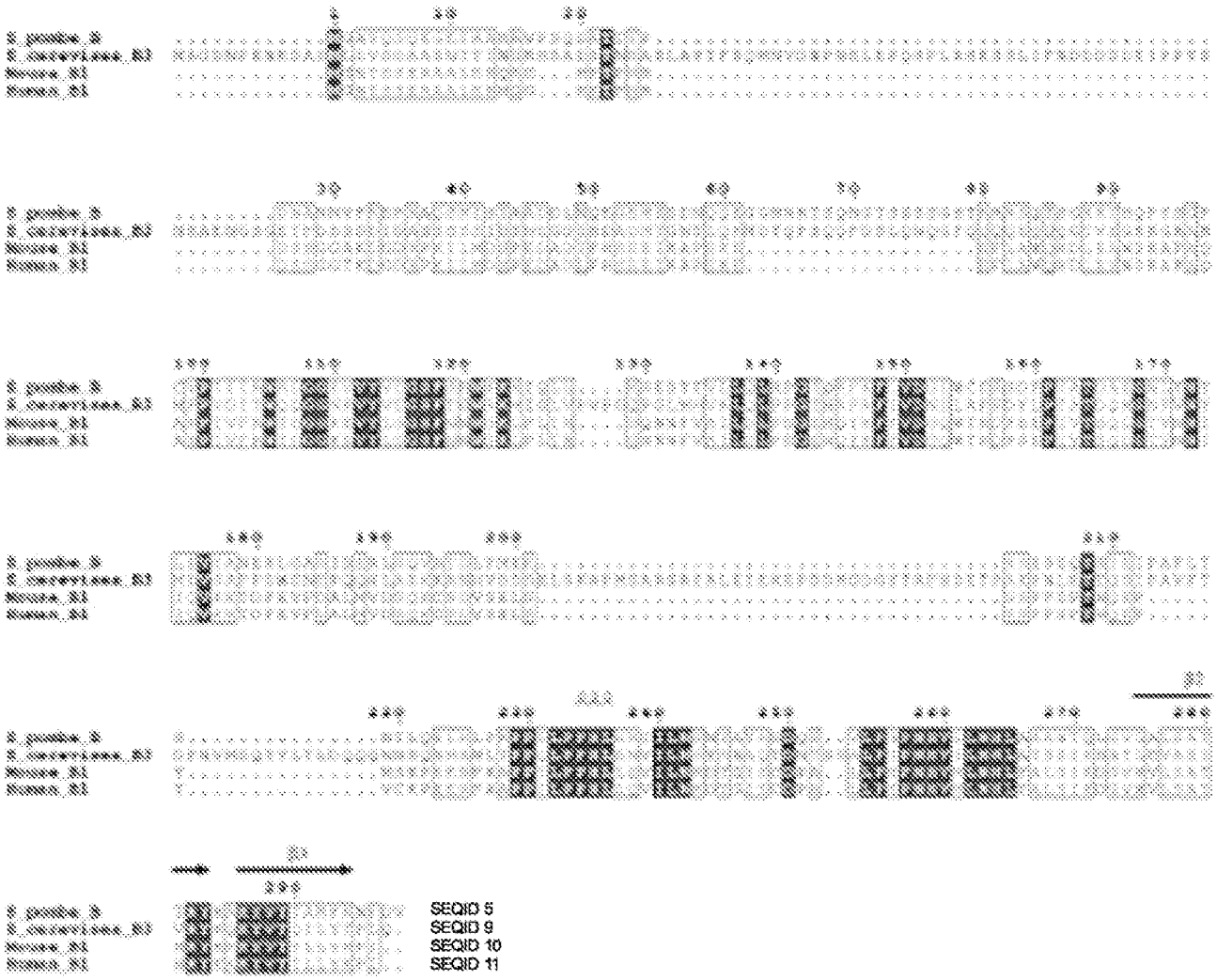


Fig. 6B

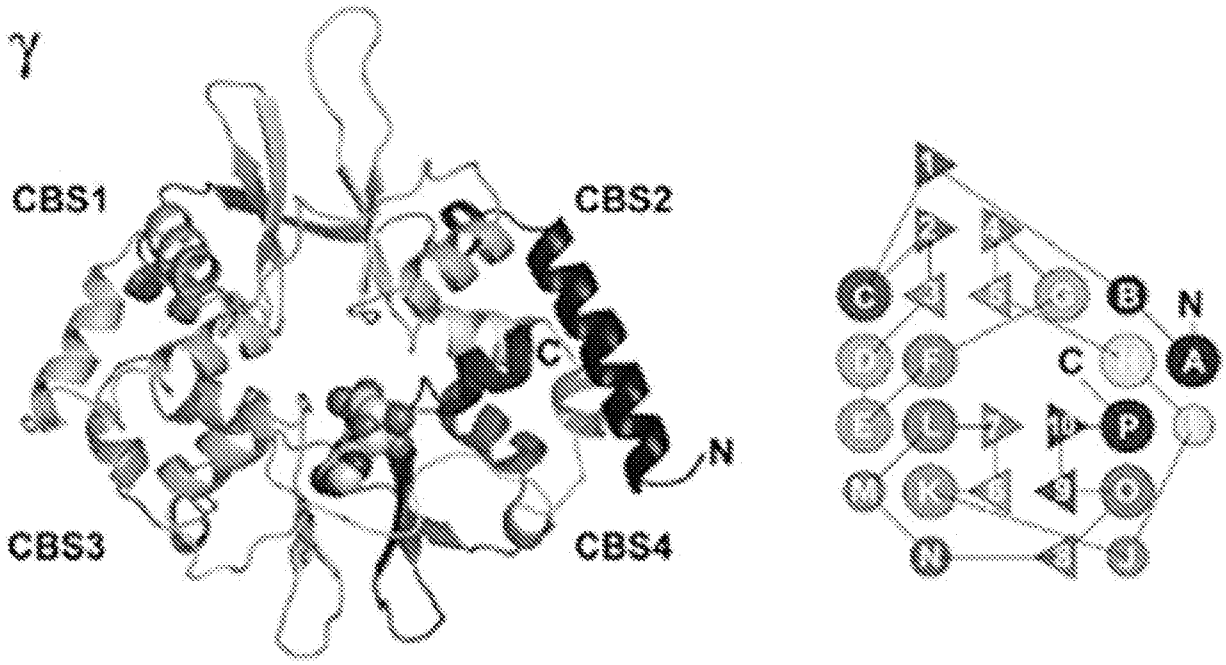


Fig. 7A

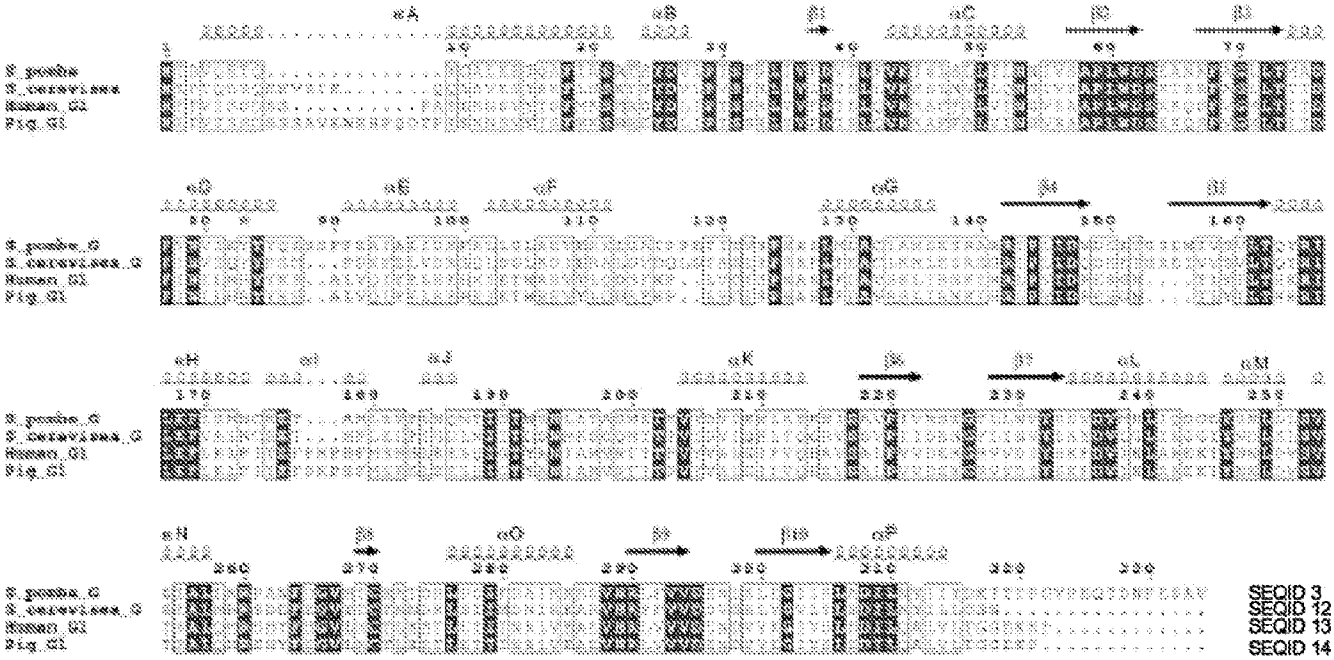


Fig. 7B

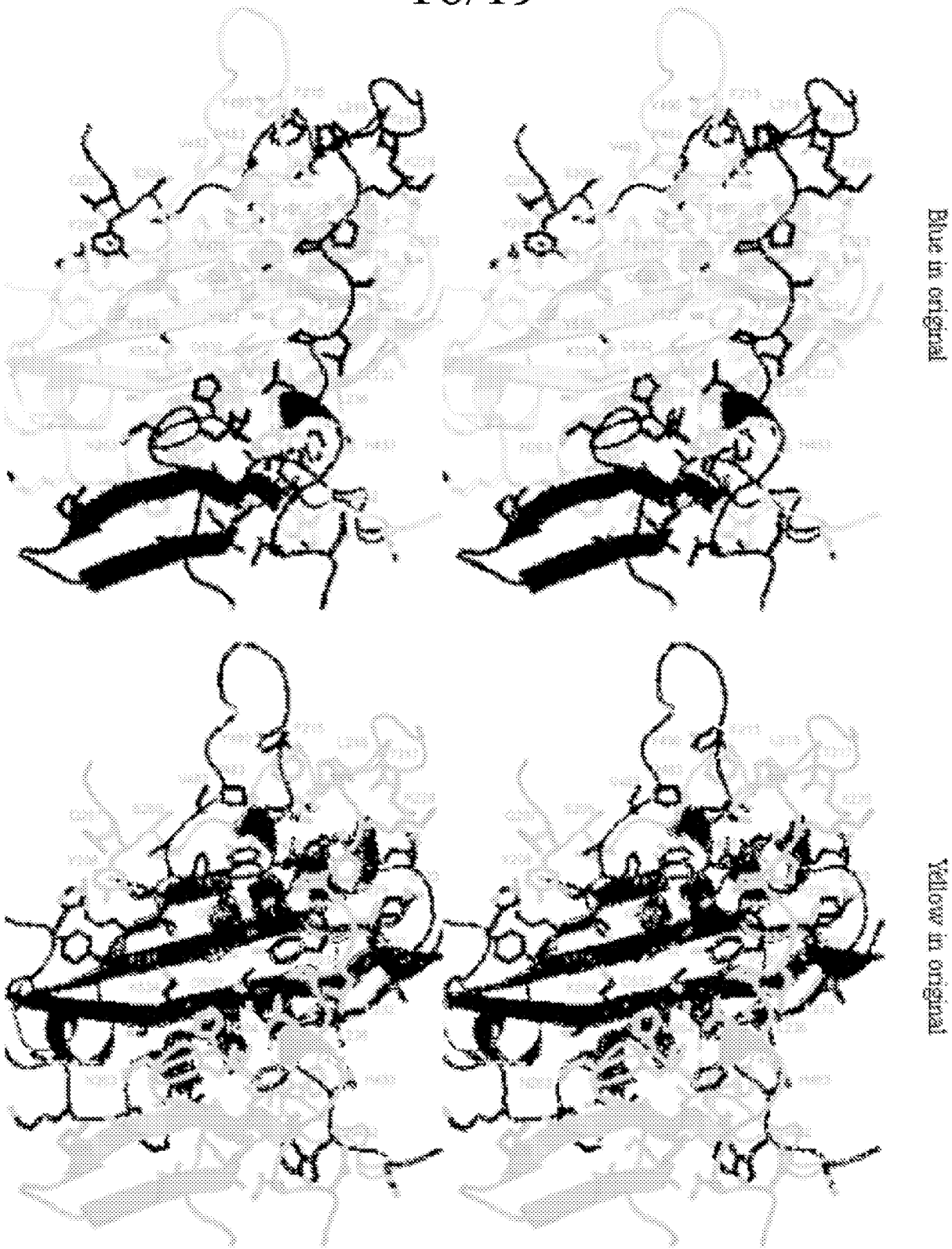


Fig. 8

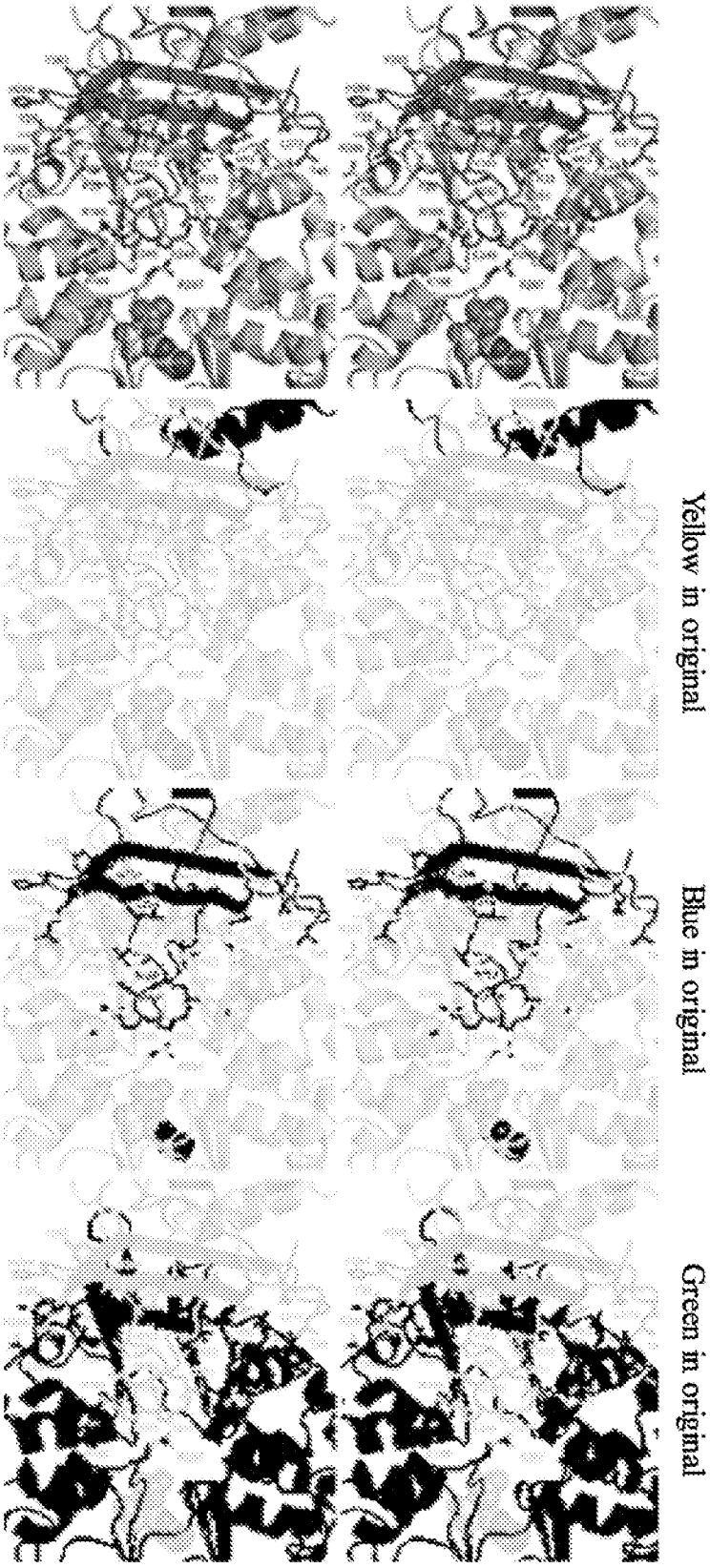


Fig. 9

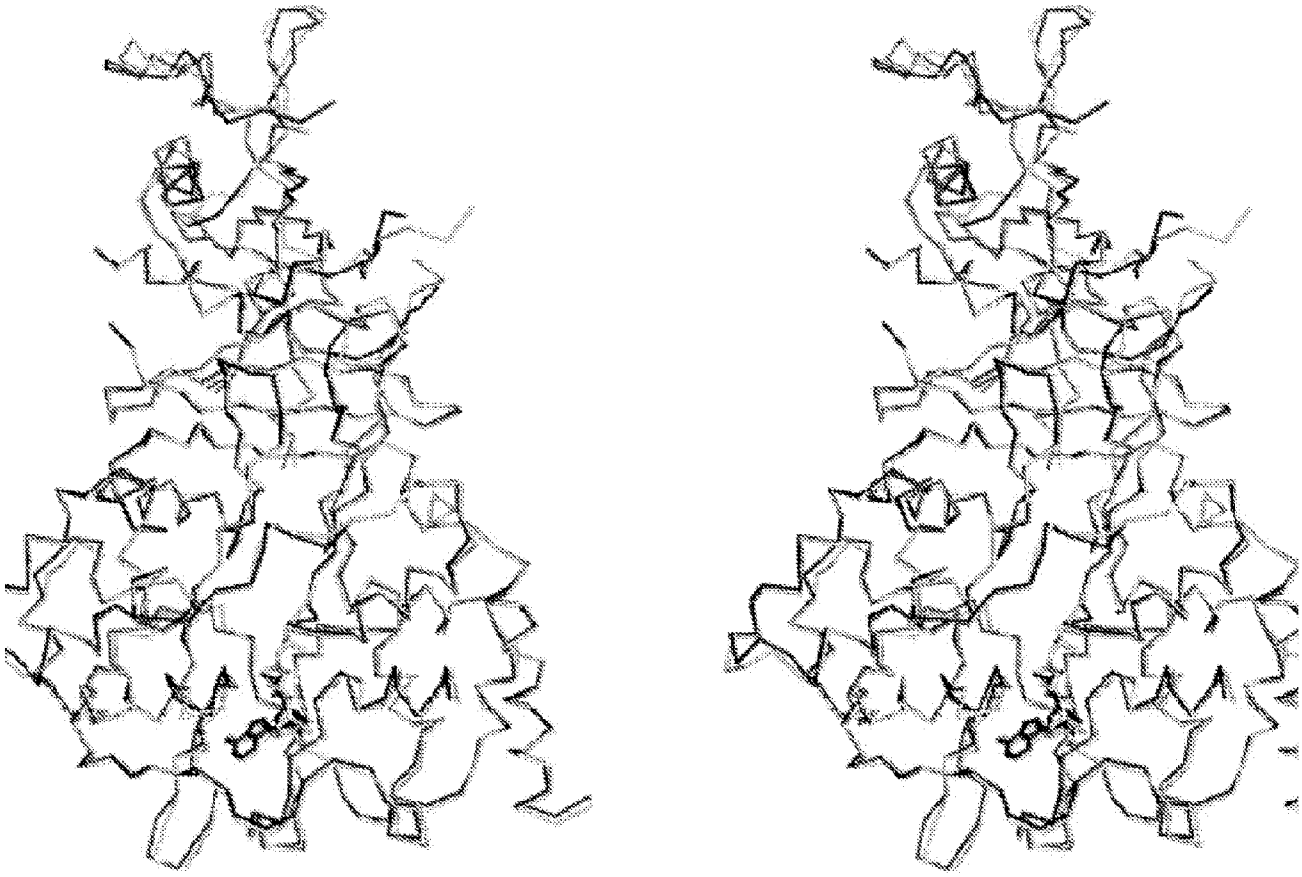


Fig. 10

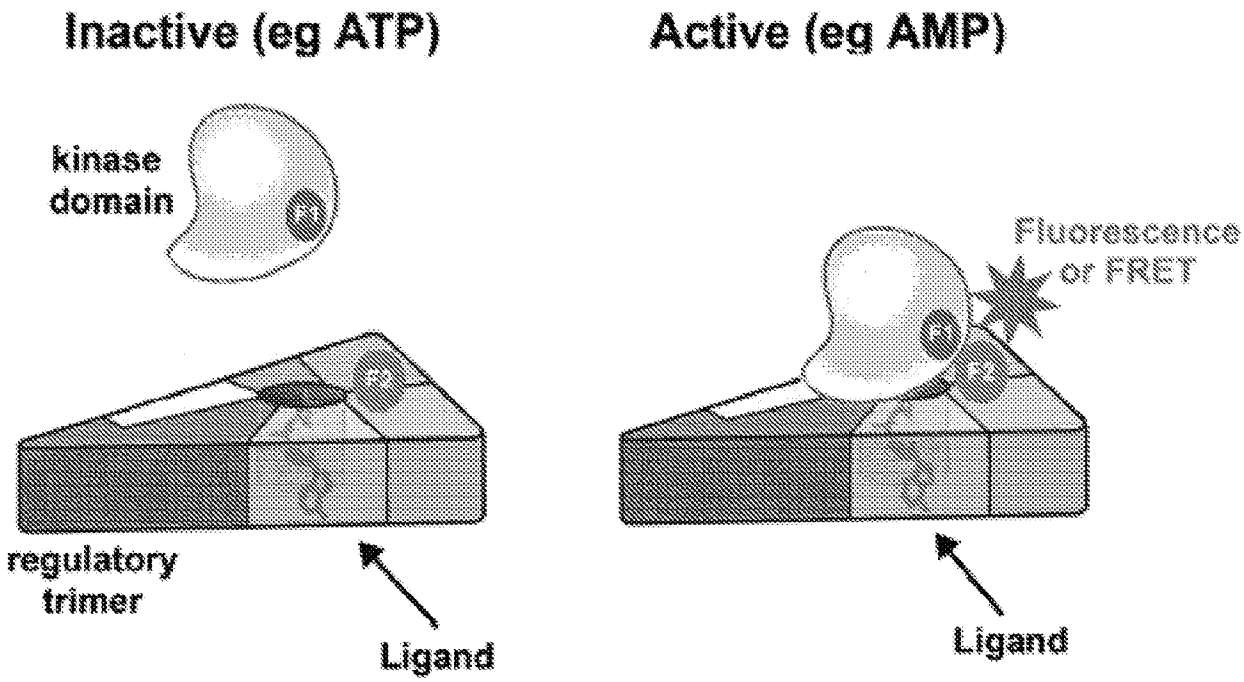

Inactive (eg ATP)
Active (eg AMP)
kinase domain
Fluorescence or FRET
regulatory trimer
Ligand
Ligand

Fig. 11