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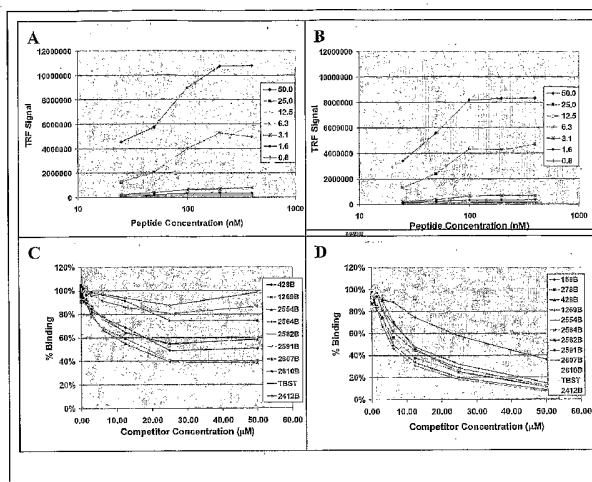
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(54) Title: ANDROGEN RECEPTOR INTERACTING PEPTIDES



ANDROGEN RECEPTOR INTERACTING PEPTIDES

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

The present invention is directed to interacting peptides, and in particular to peptides that interact with the androgen receptor ("AR").

2. Brief Description of the Related Art

10 The androgen receptor ("AR") is a ligand-activated transcriptional regulatory protein that mediates induction of male sexual development and function through its activity with endogenous androgens. Among other functions, AR plays a pivotal role in prostate homeostasis, and is clearly implicated in pathogenic states involving this organ. Because AR is a validated therapeutic target for a number of indications, it is desirable to
15 identify and characterize ligands of all types that interact with this receptor.

A number of patents exist relating to ligand modulators for androgen receptor activity. In most cases, these ligand modulators are steroidal or non-steroidal small molecules that act as agonists, partial agonists, and antagonists for the androgen receptor (AR). For example, U.S. Patent No. 6,462,038 issued to Higuchi et al. discloses non-
20 steroidal compounds that are modulators (i.e. agonists and antagonists) of androgen receptors, and to methods for the making and use of such compounds. U.S. Patent No. 6,492,554 issued to Dalton et al. discloses androgen receptor modulator compounds having in-vivo androgenic and anabolic activity of a nonsteroidal ligand for the androgen receptor. U.S. Patent No. 6,448,405 to Jones et al., also discloses non-steroidal
25 compounds that are modulators (i.e. agonists and antagonists) of steroid receptors, including the androgen receptor.

Peptides have been shown to be useful in a variety of ways in the biological and therapeutic arts. Therapeutic peptides, in particular, are becoming a part of the standard regimen for treating conditions such as prostate cancer, endometriosis, and acromegaly.
30 Therapeutic peptides may be used to treat a variety of diseases including HIV, hepatitis B, and various types of cancer. For example, U.S. Patent No. 6,465,431 to Thorn et al. discloses a therapeutic peptide consisting of fragments of troponin C, troponin I, troponin

T, which are effective to inhibit angiogenesis. Chang and McDonnell (Mol Endocrinol. 16:647-660 (2002) recently published a study showing ligand-dependent changes in AR structure using peptide probes. However, the disclosed peptides were identified from selections with estrogen receptor and found to cross react with AR. Other investigators have used peptides corresponding to AR interacting proteins to show that segments of these proteins are capable of binding to AR. However, therapeutic peptides for treatment of AR or AR-targeted diseases have not been heretofore identified.

Identification of peptides that bind to AR are desirable to understanding its mode of action and as potential modulators of AR activity. Accordingly, there is a need in the art to identify and provide peptides that bind to the androgen receptor, and that could influence the response of AR and be utilized as therapeutic treatments for AR-based diseases. The present invention is believed to be an answer to that need.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a polypeptide that binds to the androgen receptor, the polypeptide comprising the amino acid sequence Ar-X-X-Z-Ar, wherein Ar is an aromatic amino acid; X is any amino acid; and Z is a hydrophobic amino acid (Ψ) or an aromatic amino acid (Ar).

In another aspect, the present invention is directed to a polypeptide that binds to the androgen receptor, the polypeptide comprising the amino acid sequence Ser- β -Ar-X- Δ - Ψ -Ar, wherein Ar is an aromatic amino acid; X is any amino acid; Ψ is a hydrophobic amino acid; β is a basic amino acid; and Δ is an acidic amino acid.

In another aspect, the present invention is directed to a polypeptide that binds to the androgen receptor, the polypeptide comprising the amino acid sequence Ser- β -Ar- Δ -X- Ψ -Ar, wherein Ar is an aromatic amino acid; X is any amino acid; Ψ is a hydrophobic amino acid; β is a basic amino acid; and Δ is an acidic amino acid.

In another aspect, the present invention is directed to a polypeptide that binds to the androgen receptor, the polypeptide comprising the amino acid sequence Ser-X-Ar-X-X- Ψ -Ar, wherein Ar is an aromatic amino acid; X is any amino acid; and Ψ is a hydrophobic amino acid.

In other aspects, the present invention is directed to methods of analyzing the surface conformation of a protein using one or more of the above polypeptide sequences;

methods of identifying modulators of protein function using one or more of the above polypeptide sequences; pharmaceutical compositions comprising a pharmaceutically acceptable carrier and one or more of the above polypeptide sequences; and methods of treatment and/or diagnosis of diseases utilizing the above peptides or polypeptides.

5 These and other aspect will be described in more detail in the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The invention will be more fully understood from the following detailed description taken in conjunction with the accompanying figures in which:

 Figure 1, Panel A, shows a binding curve of peptide 2554 concentration versus fluorescence signal at various concentrations of androgen receptor;

 Figure 1, Panel B, shows a binding curve of peptide 2582 concentration versus fluorescence signal at various concentrations of androgen receptor;

15 Figure 1, Panel C, shows the results of a competition assay between peptide 2554 and various competitor peptides;

 Figure 1, Panel D, shows the results of a competition assay between peptide 2582 and various competitor peptides;

20 Figure 2, Panel A, shows the binding curves of eight (8) peptides to androgen receptor with increasing concentrations of dihydrotestosterone; and

 Figure 2, Panel B, shows the binding curves of eight (8) peptides to androgen receptor with increasing concentrations of cyproterone acetate.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a family of peptides that are capable of binding to the androgen receptor (AR). As defined herein, a peptide that binds to AR refers to a peptide that can interact with AR (or one or more of its domains) as monitored by standard methods such as: two-hybrid interaction in cells using a transcriptional reporter; modulation of AR activities such as AR mediated transcription, interaction with proteins, cellular localization, secondary protein modifications (phosphorylation, sumoylation, ubiquitinylation, etc.); co-immunoprecipitation (or modulation of such); direct interaction (FRET, BiaCore analysis, and the like); co-crystallization; or other methods well known in the art.

The peptides of the present invention share several sequence and structural motifs that permit members of the peptide families to bind to the androgen receptor and possibly modulate its activity. The general sequence for this family of peptides is

Ar-X-X-Z-Ar

wherein Ar is an aromatic amino acid (e.g., tryptophan, phenylalanine, tyrosine); X is any amino acid; and Z is a hydrophobic amino acid (Ψ) or an aromatic amino acid (Ar).

Accordingly, one preferred embodiment of the peptides of the present invention include a family of peptides having the general sequence

Ar-X-X- Ψ -Ar

wherein Ar is an aromatic amino acid (e.g., tryptophan, phenylalanine, and tyrosine); X is any amino acid; and Ψ is a hydrophobic amino acid (e.g., valine, leucine, isoleucine, methionine, and the like). Examples of sequences having this general sequence include (using single-letter abbreviations for the amino acids):

WQALF (SEQ ID NO:1)

FAALW (SEQ ID NO:2)

WAGLW (SEQ ID NO:3)

FQALF (SEQ ID NO:4)

Representative amino acid sequences containing these general sequences include

SRWQALFDDGTDTSR (SEQ ID NO:5)

SSKFAALWDPPKLSR (SEQ ID NO:6)

SSAGGHGKNKWAGLWRESR (SEQ ID NO:7)

SSTGIQSKFQALFSR (SEQ ID NO:8)

5 In another preferred embodiment, the peptides of the present invention have the general sequence

$$\text{Ar-X-X-Ar-Ar}$$

wherein Ar is an aromatic amino acid (e.g., tryptophan, phenylalanine, tyrosine), and X is any amino acid. Examples sequences of having this general sequence include:

10 FQNFF (SEQ ID NO:9)
FHSFW (SEQ ID NO:10)
FADFF (SEQ ID NO:11)
FAKFW (SEQ ID NO:12)

Representative amino acid sequences containing these general sequences include

15 SSFQNFFAAPYMESPLSSR (SEQ ID NO:13)
SRFHSEFWVPESWEAKVSR (SEQ ID NO:14)
SRFADFFRNEGLGSR (SEQ ID NO:15)
SSYNSSNGKFAKFWPTYSR (SEQ ID NO:16)

20 In another embodiment, another family of peptides that bind to the androgen receptor has the general amino acid sequence

$$\text{Ser-}\beta\text{-Ar-X-}\Delta\text{-}\Psi\text{-Ar}$$

wherein Ar is an aromatic amino acid; X is any amino acid; Ψ is a hydrophobic amino acid; β is a basic amino acid (e.g., lysine, arginine, histidine, and the like); and Δ is an
25 acidic amino acid (e.g., aspartic acid, glutamic acid, and the like). Examples sequences of having this general sequence include:

30 SKFYELF (SEQ ID NO:17)
SRFADIF (SEQ ID NO:18)
SRFSELF (SEQ ID NO:19)
SRFAELW (SEQ ID NO:20)

Representative amino acid sequences containing these general sequences include

SSKFYELFRNVNLSR (SEQ ID NO:21)

SRFADIFREAQQESR (SEQ ID NO:22)

SRFSELFISPPVPDGLSR (SEQ ID NO:23)

5 SRFAELWEMDWGELVDPSR (SEQ ID NO:24)

In another embodiment, another family of peptides that bind to the androgen receptor has the general amino acid sequence

Ser- β -Ar- Δ -X- Ψ -Ar

10 wherein Ar is an aromatic amino acid; X is any amino acid; Ψ is a hydrophobic amino acid; β is a basic amino acid; and Δ is an acidic amino acid. Examples sequences of having this general sequence include:

SRFDSL (SEQ ID NO:25)

SRFEHLW (SEQ ID NO:26)

15 SRWESLW (SEQ ID NO:27)

SRFEALY (SEQ ID NO:28)

Representative amino acid sequences containing these general sequences include

SRFDSLFGVWAGGEAMGSR (SEQ ID NO:29)

20 SRFEHLWKYELTDSR (SEQ ID NO:30)

SSRWESLWTGEANRSESR (SEQ ID NO:31)

SRFEALYLDRVTGLHTDTSR (SEQ ID NO:32)

25 In another embodiment, another family of peptides that bind to the androgen receptor has the general amino acid sequence

Ser-X-Ar-X-X- Ψ -Ar

wherein Ar is an aromatic amino acid; X is any amino acid; and Ψ is a hydrophobic amino acid. Examples sequences of having this general sequence include:

SLFQTM (SEQ ID NO:33)

30 SPFRSLF (SEQ ID NO:34)

SPFYTLF (SEQ ID NO:35)

SLFANRF (SEQ ID NO:36)

Representative amino acid sequences containing these general sequences include

SSNSLFQTMYLQGANFSSR (SEQ ID NO:37)

SSSPFRSLFSNGDSR (SEQ ID NO:38)

SSPFYTLFAPFPTTSPVSR (SEQ ID NO:39)

5 SSLFANRFFESVCVGGGLCSR (SEQ ID NO:40)

Any of the peptides or polypeptides of the present invention may be labeled with a detectable label, for example, of radiolabels, fluorophores, chromophores, enzymes, and combinations thereof.

10 In addition to the above peptides, the present invention is also directed to a polypeptide that comprises at least 50% amino acid sequence identity to one or more of the disclosed polypeptides. The present invention is also directed to a peptide or polypeptide that binds to the androgen receptor, wherein that binding is competitively inhibited by one or more of the disclosed peptides or polypeptides. The present invention
15 is also directed to a chimeric protein comprising one or more of the peptides or polypeptides disclosed herein, and at least a portion of a filamentous phage protein that is sufficient for integration of the chimeric protein into the coat of phage particles so as to display the polypeptide. The present invention is also directed to a filamentous phage displaying a peptide or polypeptide disclosed herein.

20 The above peptides may be isolated by themselves or in any combination. In addition, the sequences may have additional amino acids attached to N-terminal or C-terminal portions of the sequence. Alternatively, the peptides may be part of a larger polypeptide or protein. The peptides may also form all or part of a peptidomimetic structure (e.g., non-natural peptides, cyclic peptides, peptide analogs, and constrained
25 peptides).

In addition to other uses, the peptides and polypeptides of the present invention may be used in biological research and as therapeutics. The peptides and polypeptides of the present invention can be utilized in multiple formats ranging from in vitro to cell culture systems, and may be used to identify novel AR modulators that affect AR
30 mediated processes. The peptides and polypeptides of the present invention are also useful as probes to determine different surface conformations induced by AR modulators, as ligands in the crystalization of AR, and as therapeutic agents to disrupt or enhance AR

activity. Procedures for performing these operations are well known to those of skill in the art (See, Bledsoe, R. K. et al. *Cell* **110**:93-105 (2002); Shiau, A. K. et al. *Cell* **95**:927-37 (1998); Stehlin, C. et al. *EMBO J* **20**:5822-31 (2001); Paige, L. A. et al. *Proc Natl Acad Sci U S A* **96**:3999-4004 (1999); Chang, C. et al. *Mol Cell Biol* **19**:8226-39 (1999);
5 Wijayaratne, A. L. et al. *Endocrinology* **140**:5828-40 (1999); Norris, J. D. et al. *Science* **285**:744-6 (1999); Hyde-DeRuyscher, R. et al. *Chem Biol* **7**:17-25. (1999), and International Patent Application WO 98/19162, all of which are incorporated by reference in their entireties).

The peptides of the present invention are also useful as a panel for in vitro and in
10 vivo classification of compounds. See, for example, International Patent Application WO 99/54728, hereby incorporated by reference in its entirety, which discloses use of peptides (termed "BioKeys") as probes for use in an investigation of alteration of receptor conformation. The peptides of the present invention may also be used in a competitive displacement assay to identify modulators of receptor activity in a high-throughput screen;
15 to fingerprint modulators of receptor activity and classify them as agonists or antagonists of receptor activity; or to identify the natural ligands of orphan receptors.

With respect to use of the peptides and polypeptides as therapeutic agents, the peptides and polypeptides of the present invention are preferably part of pharmaceutical compositions generally comprising one or more of the peptides or polypeptides in
20 combination with a pharmaceutically acceptable carrier. Such a pharmaceutical composition is preferably administered internally, e.g., intravenously, in the form of conventional pharmaceutical preparations, for example in conventional enteral or parenteral pharmaceutically acceptable excipients containing organic and/or inorganic inert carriers, such as water, gelatin, lactose, starch, magnesium stearate, talc, plant oils,
25 gums, alcohol, Vaseline, or the like. The pharmaceutical preparations can be in conventional solid forms, for example, tablets, dragees, suppositories, capsules, or the like, or conventional liquid forms, such as suspensions, emulsions, or the like. If desired, they can be sterilized and/or contain conventional pharmaceutical adjuvants, such as preservatives, stabilizing agents, wetting agents, emulsifying agents, buffers, or salts used
30 for the adjustment of osmotic pressure. The pharmaceutical compositions may also contain other therapeutically active materials.

The pharmaceutical composition of the invention should include an amount of the peptide(s) or polypeptide(s) of the invention effective for desired activity. The effective dosage will depend on the activity and toxicity of the particular peptide or peptides employed and is thus within the ordinary skill of the art to determine for any particular host mammal or other host organism. Suitable dosages may be, for example, in the range of about 0.5-100 mg per kg for a human being. Alternatively, the claimed peptides may be used to control the androgen receptor in vitro or may be used as a modulating agent in nonhuman mammals.

With regard to therapeutic agents, the present invention is also directed to a method of diagnosing a disease in a patient characterized by abnormal levels of activation of androgen receptor (AR) comprising (1) providing a sample of body fluid or tissue of said patient; (2) administering a diagnostically effective amount of the above pharmaceutical composition, and (3) assaying the amount of activated AR in said body fluid or tissue of said patient. The present invention is also directed to a method for treating a patient suffering from a disease characterized by abnormal levels of activation of androgen receptor (AR), comprising administering to said patient a therapeutically effective amount of the above pharmaceutical composition. One disease characterized by abnormal levels of activation of AR is prostate cancer.

While preliminary screening assays are used to determine the activity of a compound of uncertain activity, diagnostic assays employ a binding molecule of known binding activity, or a conjugate or derivative thereof, as a diagnostic reagent. For the purpose of the discussion of diagnostic methods and agents which follows, the "binding molecule" may be a peptide, peptoid, peptidomimetic or other analogue of the present invention, or an oligonucleotide of the present invention, which binds the analyte or a binding partner of the analyte. The analyte is a target protein.

In vitro assays may be diagnostic assays (using a known binding molecule to detect or measure an analyte) or screening assays (determining whether a potential binding molecule in fact binds a target). The format of these two types of assays is very similar and, while the description below refers to diagnostic assays for analytes, it applies, mutatis mutandis, to the screening of molecules for binding to targets. The in vitro assays of the present invention may be applied to any suitable analyte-containing sample, and may be qualitative or quantitative in nature.

In order to detect the presence, or measure the amount, of an analyte, the assay must provide for a signal producing system (SPS) in which there is a detectable difference in the signal produced, depending on whether the analyte is present or absent (or, in a quantitative assay, on the amount of the analyte). This signal is, or is derived from, one or
5 more observable raw signals.

The raw signal for a particular state (e.g., presence or amount of analyte) is the level of an observable parameter, or of a function dependent on the level(s) of one or more observable parameters. The signal is a difference in raw signals, depending on the states to be differentiated by the assay. The signal may be direct (increased if the amount of
10 analyte increases) or inverse (decreased raw signal if the amount of analyte increases). The signal may be absolute (in one state, there is no detectable raw signal at all) or relative (a change in the level of the raw signal, or of the rate of change in the level of the raw signal). The signal may be discrete (yes or no, depending on the level of the raw signal relative to some threshold) or continuous in value. The signal may be simple (based on a
15 single raw signal) or composite (based on a plurality of raw signals). The detectable raw signal may be one which is visually detectable, or one detectable only with instruments. Possible raw signals include production of colored or luminescent products, alteration of the characteristics (including amplitude or polarization) of absorption or emission of radiation by an assay component or product, and precipitation or agglutination of a
20 component or product. The raw signal may be monitored manually or automatically.

The component of the signal producing system which is most intimately associated with the diagnostic reagent is called the "label". A label may be, e.g., a radioisotope, a fluorophore, an enzyme, a co-enzyme, an enzyme substrate, an electron-dense compound, or an agglutinable particle. One diagnostic reagent is a conjugate, direct or indirect, or
25 covalent or noncovalent, of a label with a binding molecule of the invention.

The radioactive isotope can be detected by such means as the use of a gamma counter or a scintillation counter or autoradiography. Isotopes which are particularly useful for the purpose of the present invention are ^3H , ^{125}I , ^{131}I , ^{35}S , ^{14}C , and, preferably,
30 ^{125}I .

It is also possible to label a compound with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent

labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, fluorescence-emitting metals such as ^{125}Eu , or others of the lanthanide series, may be attached to the binding protein using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediamine-tetraacetic acid (EDTA).

The binding molecules also can be detectably labeled by coupling to a chemiluminescent compound. The presence of the chemiluminescent compound is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction after a suitable reactant is provided. Examples of particularly useful chemiluminescent labeling compounds are luminol, isolumino, thermotropic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the binding molecule. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Enzyme labels, such as horseradish peroxidase and alkaline phosphatase, are preferred. When an enzyme label is used, the signal producing system must also include a substrate for the enzyme. If the enzymatic reaction product is not itself detectable, the SPS will include one or more additional reactants so that a detectable product appears.

Assays may be divided into two basic types, heterogeneous and homogeneous. In heterogeneous assays, the interaction between the affinity molecule and the analyte does not affect the label, hence, to determine the amount or presence of analyte, bound label must be separated from free label. In homogeneous assays, the interaction does affect the activity of the label, and therefore analyte levels can be deduced without the need for a separation step.

In general, a target-binding molecule of the present invention may be used diagnostically in the same way that a target-binding antibody is used. Thus, depending on the assay format, it may be used to assay the target, or by competitive inhibition, other substances which bind the target. The sample will normally be a biological fluid, such as

blood, urine, lymph, semen, milk, or cerebrospinal fluid, or a fraction or derivative thereof, or a biological tissue, in the form of, e.g., a tissue section or homogenate. However, the sample conceivably could be (or derived from) a food or beverage, a pharmaceutical or diagnostic composition, soil, or surface or ground water. If a biological
5 fluid or tissue, it may be taken from a human or other mammal, vertebrate or animal, or from a plant. The preferred sample is blood, or a fraction or derivative thereof.

In one embodiment, the binding molecule is insolubilized by coupling it to a macromolecular support, and target in the sample is allowed to compete with a known quantity of a labeled or specifically labelable target analogue. (The conjugate of the
10 binding molecule to a macromolecular support is another diagnostic agent within the present invention.) The "target analogue" is a molecule capable of competing with target for binding to the binding molecule, and the term is intended to include target itself. It may be labeled already, or it may be labeled subsequently by specifically binding the label to a moiety differentiating the target analogue from authentic target. The solid and liquid
15 phases are separated, and the labeled target analogue in one phase is quantified. The higher the level of target analogue in the solid phase, i.e., sticking to the binding molecule, the lower the level of target analyte in the sample.

In a "sandwich assay", both an insolubilized target-binding molecule, and a labeled target-binding molecule are employed. The target analyte is captured by the insolubilized
20 target-binding molecule and is tagged by the labeled target-binding molecule, forming a tertiary complex. The reagents may be added to the sample in either order, or simultaneously. The target-binding molecules may be the same or different, and only one need be a target-binding molecule according to the present invention (the other may be, e.g., an antibody or a specific binding fragment thereof). The amount of labeled target-
25 binding molecule in the tertiary complex is directly proportional to the amount of target analyte in the sample.

The two embodiments described above are both heterogeneous assays. However, homogeneous assays are conceivable. The key is that the label be affected by whether or not the complex is formed.

30 A label may be conjugated, directly or indirectly (e.g., through a labeled anti-target-binding molecule antibody), covalently (e.g., with SPDP) or noncovalently, to the target-binding molecule, to produce a diagnostic reagent. Similarly, the target binding

molecule may be conjugated to a solid-phase support to form a solid phase ("capture") diagnostic reagent. Suitable supports include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to its target. Thus the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc.

Analyte-binding molecules can be used for *in vivo* imaging. Radio-labelled binding molecule may be administered to the human or animal subject. Administration is typically by injection, e.g., intravenous or arterial or other means of administration in a quantity sufficient to permit subsequent dynamic and/or static imaging using suitable radio-detecting devices. The preferred dosage is the smallest amount capable of providing a diagnostically effective image, and may be determined by means conventional in the art, using known radio-imaging agents as a guide.

Typically, the imaging is carried out on the whole body of the subject, or on that portion of the body or organ relevant to the condition or disease under study. The radio-labelled binding molecule has accumulated. The amount of radio-labelled binding molecule accumulated at a given point in time in relevant target organs can then be quantified.

A particularly suitable radio-detecting device is a scintillation camera, such as a gamma camera. A scintillation camera is a stationary device that can be used to image distribution of radio-labelled binding molecule. The detection device in the camera senses the radioactive decay, the distribution of which can be recorded. Data produced by the imaging system can be digitized. The digitized information can be analyzed over time discontinuously or continuously. The digitized data can be processed to produce images, called frames, of the pattern of uptake of the radio-labelled binding protein in the target organ at a discrete point in time. In most continuous (dynamic) studies, quantitative data is obtained by observing changes in distributions of radioactive decay in target organs over time. In other words, a time-activity analysis of the data will illustrate uptake through clearance of the radio-labelled binding molecule by the target organs with time.

Various factors should be taken into consideration in selecting an appropriate radioisotope. The radioisotope must be selected with a view to obtaining good quality resolution upon imaging, should be safe for diagnostic use in humans and animals, and should preferably have a short physical half-life so as to decrease the amount of radiation received by the body. The radioisotope used should preferably be pharmacologically inert, and, in the quantities administered, should not have any substantial physiological effect.

The binding molecule may be radio-labelled with different isotopes of iodine, for example ^{123}I , ^{125}I , or ^{131}I (see for example, U. S. Patent 4,609,725). The extent of radio-labeling must, however be monitored, since it will affect the calculations made based on the imaging results (i.e., a diiodinated binding molecule will result in twice the radiation count of a similar monoiodinated binding molecule over the same time frame).

In applications to human subjects, it may be desirable to used radioisotopes other than ^{125}I for labeling in order to decrease the total dosimetry exposure of the human body and to optimize the detectability of the labeled molecule (though this radioisotope can be used if circumstances require). Ready availability for clinical use is also a factor.

Accordingly, for human applications, preferred radio-labels are for example, $^{99\text{m}}\text{Tc}$, ^{67}Ga , ^{68}Ga , ^{90}Y , ^{111}In , $^{113\text{m}}\text{In}$, ^{123}I , ^{186}Re , ^{188}Re , or ^{211}At .

The radio-labelled binding molecule may be prepared by various methods. These include radio-halogenation by the chloramines - T method or the lactoperoxidase method and subsequent purification by HPLC (high pressure liquid chromatography), for example as described by J. Gutkowska et al. in "Endocrinology and Metabolism Clinics of America" (1987) 16:183. Other known method of radio-labelling can be used, such as IODOBEADSTM.

There are a number of different methods of delivering the radio-labelled binding molecule to the end-user. It may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. If the molecule is digestible when administered orally, parenteral administration, e.g., intravenous, subcutaneous, or intramuscular, would ordinarily be used to optimize absorption.

The binding molecules of the present invention may also be used to purify target from a fluid, e.g., blood. For this purpose, the target-binding molecule is preferable

immobilized on a solid-phase support. Such supports include those already mentioned as useful in preparing solid phase diagnostic reagents.

Peptides, in general, can be used as molecular weight markers for reference in the separation or purification of peptides by electrophoresis or chromatography. In many instances, peptides may need to be denatured to serve as molecular weight markers. A second general utility for peptides is the use of hydrolyzed peptides as a nutrient source. Hydrolyzed peptide are commonly used as a growth media component for culturing microorganisms, as well as a food ingredient for human consumption. Enzymatic or acid hydrolysis is normally carried out either to completion, resulting in free amino acids, or partially, to generate both peptides and amino acids. However, unlike acid hydrolysis, enzymatic hydrolysis (proteolysis) does not remove non-amino acid functional groups that may be present. Peptides may also be used to increase the viscosity of a solution.

The peptides of the present invention may be used for any of the foregoing purposes, as well as for therapeutic and diagnostic purposes.

EXAMPLES

The present invention is further described in detail by means of the following Examples. All parts and percentages are by weight and all temperatures are in degrees Celsius unless explicitly stated otherwise.

Examples 1-62: Identification Of Androgen Receptor Interacting Peptides

Any method of identifying peptides, and particularly those methods that select for peptides that bind at biologically relevant sites of the target receptor, may be used in the present invention. See, for example, Hyde-DeRuyscher, R. et al. *Chem Biol* 7:17-25. (1999), herein incorporated by reference in its entirety.

In the present invention, to identify peptides that interact with the androgen receptor, phage display techniques were performed using the ligand binding domain of the androgen receptor. Affinity selection of phage-displayed peptides was carried out basically as described by Sparks et. al. (Sparks AB, Adey NB, Cwirla S, Kay BK. (1996) in *Phage Display of Peptides and Proteins, A Laboratory Manual*, eds. Kay, B. K., Winter, J. & McCafferty, J. (Academic, San Diego), pp. 227-253), which is herein incorporated by reference in its entirety. Briefly, biotinylated target protein was immobilized in a

streptavidin-coated microtiter well. M13 phage particles distributed among 21 libraries displaying a total of greater than 2×10^{10} different random or biased amino acid sequences were added to the immobilized target protein and incubated for 3 hrs at 25°C. Unbound phage were washed away and the bound phage were eluted using pH 2 glycine. The
5 eluted phage were amplified by infecting E. coli cells. The amplified phage were then added to immobilized target protein and the cycle of affinity selection was repeated. Enrichment of phage displaying target-specific peptides was monitored after each round of affinity selection using an anti-M13 antibody conjugated to horseradish peroxidase in an ELISA-type assay. Pools of phage enriched for target-specific peptides were plated for
10 individual plaques. The plaques were picked, the phage amplified, and the phage tested for target-specific binding versus non-specific binding to various control proteins such as hexokinase, alcohol dehydrogenase, β -galactosidase, and streptavidin. DNA was prepared from target-specific phage, the DNA sequence of the peptide-encoding region was determined, and the peptide sequence was deduced. For each target, the peptide sequences
15 were compared and aligned for common motifs. Phage displaying AR-specific peptides were analyzed for their relative binding affinity for the target protein. The phage were serially diluted over a 100-fold range and tested for binding to the target protein using a phage ELISA assay. Phage that gave a higher ELISA signal at a lower dilution displayed peptides with a relatively higher affinity for the target protein. Based on their relative
20 affinity for AR, peptides sequences from different sequence clusters were selected for synthesis. The peptides were synthesized with a 5 amino acid linker sequence and a C-terminal biotin.

Additional methods of identifying peptides that bind to the androgen receptor are known in the art and may also be used in the method of the present invention. One useful
25 method is described in International Patent Application Publication No. WO 98/19162 mentioned above. Briefly, the method disclosed in this publication is directed to the identification of compounds in a compound library, including biopolymers such as peptides, that mediate the biological activity of a target receptor protein, even when the ligands that mediate that activity through binding to that receptor are not already known.
30 Such compounds can then be used as "drug leads", i.e., used as a starting point for the design of analogues which can in turn be tested for activity. As disclosed in this publication, it is believed that those members of a combinatorial library, especially a

biopolymer library, which bind to a target protein having a biologically significant binding activity will bind preferentially to the sites at which the target protein interacts with the natural binding partners which mediate its biological activity, as opposed to randomly, with equal probability, over the entire surface of the target protein. The method described in this publication comprises three general steps: (1) Screen at least one potential surrogate combinatorial library for members (preferably peptides or nucleic acids) binding to the target protein and hence capable of use as surrogates for the unknown ligand in steps (2) and (3); (2) Screen at least one complementary library, preferably a combinatorial library, (which is not limited to, and may not even include peptides or nucleic acids) for compounds which inhibit the binding of one or more surrogates (e.g., peptides or nucleic acids which bind to the target protein); and (3) Determine whether the inhibitory compound mediates the biological activity of the target protein.

The sequences of the identified peptides are as follows, along with their respective identification numbers.

	<u>Sequence</u>	<u>ID Number</u>
	SRWQALFDDGTDTSR	2551 (SEQ ID NO:5)
	SSKFAALWDPPKLSR	2591 (SEQ ID NO:6)
	SSAGGHGKNKWAGLWRESR	2600 (SEQ ID NO:7)
20	SSTGIQSKFQALFSR	2581 (SEQ ID NO:8)
	SSKFYELFRNVNLSR	2604 (SEQ ID NO:21)
	SSFFSDLFLLPEGRQAASR	2596 (SEQ ID NO:41)
	SRFADIFREAQQESR	2606 (SEQ ID NO:22)
	SRFSELFISPYPVPDGLSR	2597 (SEQ ID NO:23)
25	SRFAELWEMDWGELVDPSR	2602 (SEQ ID NO:24)
	SRWAEVWDDNSKVS	2587 (SEQ ID NO:42)
	SRWNDLFSLENTSARPNSR	2593 (SEQ ID NO:43)
	SRWCDLWEGRVECMKEVASR	2603 (SEQ ID NO:44)
	SSLGRQHWLELWGATDGSR	2599 (SEQ ID NO:45)
30	SRFADLFSESGSSR	2553 (SEQ ID NO:46)
	SRFLDVFAADWEAGGGASR	2569 (SEQ ID NO:47)
	SSEVTGMRFRDLFSR	2561 (SEQ ID NO:48)
	SRFSDCYKMQSDCSR	2583 (SEQ ID NO:49)
	SSSGRTSGAWERLWHGSR	2573 (SEQ ID NO:50)
35	SSMTPFEMLYINGHSRLSR	2594 (SEQ ID NO:51)
	SSMGATKWDVLWSR	2588 (SEQ ID NO:52)
	SRTSQMGAFEWAFRGAESR	2575 (SEQ ID NO:53)
	SRFDSLFGVWAGGEAMGSR	2611 (SEQ ID NO:29)
	SRFEHLWKYELTDSR	2586 (SEQ ID NO:30)
40	SSRWESLWTGEANRSESR	2598 (SEQ ID NO:31)

	SRFEALYLDRVTGLHTDTSR	2577 (SEQ ID NO:32)
	SRTSQMGAFEWAFRGAESR	2554 (SEQ ID NO:54)
	SSDSSRWELLWGRANGHSR	2563 (SEQ ID NO:55)
	SRFEWMFNLPDGNSR	2557 (SEQ ID NO:56)
5	SSRFESMWPECHRSKSCSR	2564 (SEQ ID NO:57)
	SRFGEMWPD RHSSSR	2559 (SEQ ID NO:58)
	SSIGRLMFPDLEPSRSFSR	2576 (SEQ ID NO:59)
	SRWMNMWDIADRSSR	2567 (SEQ ID NO:60)
	SRGLWDFRAMFQEEHSSR	2566 (SEQ ID NO:61)
10	SRFTEMWLAAETESR	2555 (SEQ ID NO:62)
	SRFQDMFDSFPSHEFSGSR	2571 (SEQ ID NO:63)
	SSNSLFQTMYLQGANFSSR	2595 (SEQ ID NO:37)
	SRFSAMFWANQADSR	2592 (SEQ ID NO:64)
	SRFTDMFLEETGPSR	2590 (SEQ ID NO:65)
15	SSFTNMFGQYAPLHSFDSR	2568 (SEQ ID NO:66)
	SSFERLWNNARLNSDSSR	2578 (SEQ ID NO:67)
	SSFASVFFPPLADQLGNSR	2585 (SEQ ID NO:68)
	SSFEVWSGESARSR	2558 (SEQ ID NO:69)
	SSFKNLWDAADGNSR	2552 (SEQ ID NO:70)
20	SSYWSTFWQSELNSR	2562 (SEQ ID NO:71)
	SSCASFKGLWAGMGEMGSR	2612 (SEQ ID NO:72)
	SSASFRSLFSASESR	2589 (SEQ ID NO:73)
	SSWSSLFEYDFPSTVGLSR	2601 (SEQ ID NO:74)
	SSEGQLFNRMFHHSR	2605 (SEQ ID NO:75)
25	SRLNCLFSTDGNSWVCSR	2584 (SEQ ID NO:76)
	SSSPFRSLFSNGDSR	2556 (SEQ ID NO:38)
	SSPFYTLFAPFPTTSPVSR	2572 (SEQ ID NO:39)
	SSLFANRFFESVCVGLCSR	2579 (SEQ ID NO:40)
	SSVSRGGWLGLWNNEKVSR	2574 (SEQ ID NO:77)
30	SSFQNFFAAPYMESPLSSR	2608 (SEQ ID NO:13)
	SRFHSFWEVPESWEAKVSR	2609 (SEQ ID NO:14)
	SRFADFFRNEGLGSR	2607 (SEQ ID NO:15)
	SSYNSSNGKFAKFWPTYSR	2610 (SEQ ID NO:16)
	SSNTPRFKEYFMQSR	2582 (SEQ ID NO:78)
35	SRAGKFDRFWQEESR	2560 (SEQ ID NO:79)
	SRFEAFYGVGEEGSGVSR	2570 (SEQ ID NO:80)
	SSFDKFYLDEYHGRGYQSR	2565 (SEQ ID NO:81)
	SRLSDLVKGAEQCVGCMGSR	2580 (SEQ ID NO:82)
40		

Example 63: Testing of Synthetic Peptides for to the Target and Competition with Other Peptides

The binding of target-specific, synthetic peptides was tested using a “peptide-on-plate” (POP) assay format, in which biotinylated peptide is first immobilized onto a BSA-blocked streptavidin-coated microtiter well, and then Europium-conjugated target protein

is allowed to react with the biotinylated peptide. Europium-conjugated protein that remains bound to the immobilized peptide after washing is read via time-resolved fluorescence (TRF). Peptides were initially tested for binding using varying peptide and target-protein concentrations (25-400 nM protein and 0.8-50 nM peptide). Concentrations
5 that gave the greatest signal:background ratio when the signal had not reached saturation were chosen for further assay development.

Using conditions derived from the above binding studies, synthetic peptides derived from peptides **2554** and **2582** were tested for their ability to compete with their own binding and with the binding of other peptides to AR. The test biotinylated-peptide is
10 immobilized onto a blocked streptavidin-coated microtiter well as above. Europium-conjugated AR target protein is incubated with increasing concentrations of competitor peptide. The mixture of target and competitor peptide is added to the wells containing immobilized test peptide. Retained AR target protein is monitored by time-resolved fluorescence and the amount of competition by each peptide quantitated relative to no
15 competitor peptide.

By titrating both protein and peptide concentration it was revealed that the interaction of these peptides was dose dependent (Figure 1, panels A and B). As shown in Figure 1, there is an increase in specific fluorescent signal with increasing protein and peptide. Using a single concentration of protein and either peptide, the assay was used to
20 determine if these and other synthetic peptides were capable of competing for peptide binding. Increasing concentrations of competitor peptides are capable of decreasing the specific signal derived from either 2554 or 2582 peptide binding (Figure 1, panels C and D). The data indicate that these peptides bind to a similar or overlapping site.

25 Example 64: Cell-based Peptide Binding Assays

Interactions between androgen receptor and peptides selected in phage display can be monitored *in vivo* using a mammalian two-hybrid system. In this system, androgen receptor is expressed as an in-frame, carboxy-terminal fusion to a transcriptional
30 activation domain derived from the herpes simplex virus VP16 protein. The peptide of interest is expressed as an in-frame, carboxy-terminal fusion to the yeast derived GAL4 DNA binding domain. The reporter used to monitor peptide/receptor interactions is a

luciferase gene downstream of five consensus GAL4 binding sites. Luciferase activity can be corrected for transfection efficiency by including a β -galactosidase gene driven by a CMV derived promoter.

Cells are seeded in media containing 10% fetal bovine serum to a density of 50-80% confluency in multi-well plates the day before transfection. In all steps, phenol red free media containing charcoal dextran stripped fetal bovine serum is used as essentially steroid-free media. Transfections are carried out in triplicate using lipofectamine to deliver DNA (ratio: 50 ng pCMV-bgal, 1500 ng 5xGAL-luc3, 900 ng pM-peptide, and 500 ng pVP16-receptor). The transfection media is applied for 6 hours and then removed by aspiration. Media containing test compound dilutions is applied to the cells and they incubate for 18-24 hours. The cells are washed twice with PBS and then lysed in 65 μ l of lysis buffer from Tropix Galacto Light Plus

Luciferase and β -galactosidase activities are determined by luminescence as described by the vendor (Tropix LucScreen and Galacto Light Plus). 20 μ l samples of clarified cell lysate is used in each assay and luminescence determined using an LJI Analyst. The luciferase activities are normalized to the corresponding β -galactosidase values to generate the steroid-dependent peptide/receptor interactions.

Peptide sequences corresponding to those listed above were cloned into mammalian cell expression vectors as Gal4 DNA binding domain fusions. The full length AR was cloned into a mammalian expression vector as an in frame fusion with the VP16 activation domain. Interactions between peptide and AR are monitored by the increase of a reporter gene (luciferase) under the control of Gal4 response elements. Performing transient transfections in a human hepatocyte cell line, a ligand dependent interaction between peptide and AR can be identified (Figure 2, panels A and B). The interactions between peptide and AR are dose dependent on the ligand added to the mammalian cell culture. The specific interactions between peptides and AR are dependent on the ligand that is added; DHT increases binding of all peptides except 158, whereas CPA increases binding of peptides to a different extent. This difference in binding can be attributed to different surface conformations attained by AR in the presence of the different compounds.

WHAT IS CLAIMED IS:

1. A polypeptide that binds to the androgen receptor, said polypeptide comprising the amino acid sequence Ar-X-X-Z-Ar, wherein:

- 5 Ar is an aromatic amino acid;
 X is any amino acid; and
 Z is a hydrophobic amino acid (Ψ) or an aromatic amino acid (Ar).

2. The polypeptide of claim 1, wherein Z is Ψ .

10

3. The polypeptide of claim 1, wherein Z is Ar.

4. The polypeptide of claim 1, wherein

Ar is selected from the group consisting of Tryptophan, Phenylalanine, and

15 Tyrosine;

Ψ is selected from the group consisting of Valine, Leucine, Isoleucine, and

Methionine;

β is selected from the group consisting of Lysine, Arginine, and Histidine; and

Δ is selected from the group consisting of aspartic acid and glutamic acid.

20

5. A polypeptide that binds to the androgen receptor, said polypeptide comprising the amino acid sequence Ser- β -Ar-X- Δ - Ψ -Ar, wherein:

Ar is an aromatic amino acid;

X is any amino acid;

25 Ψ is a hydrophobic amino acid;

β is a basic amino acid; and

Δ is an acidic amino acid.

6. The polypeptide of claim 5, wherein
Ar is selected from the group consisting of Tryptophan, Phenylalanine, and Tyrosine;

5 Ψ is selected from the group consisting of Valine, Leucine, Isoleucine, and Methionine;

β is selected from the group consisting of Lysine, Arginine, and Histidine; and

Δ is selected from the group consisting of aspartic acid and glutamic acid.

7. A polypeptide that binds to the androgen receptor, said polypeptide comprising the
10 amino acid sequence Ser-β-Ar-Δ-X-Ψ-Ar, wherein:

Ar is an aromatic amino acid;

X is any amino acid;

Ψ is a hydrophobic amino acid;

β is a basic amino acid; and

15 Δ is an acidic amino acid.

8. The polypeptide of claim 7, wherein

Ar is selected from the group consisting of Tryptophan, Phenylalanine, and Tyrosine;

20 Ψ is selected from the group consisting of Valine, Leucine, Isoleucine, and Methionine;

β is selected from the group consisting of Lysine, Arginine, and Histidine; and

Δ is selected from the group consisting of aspartic acid and glutamic acid.

25 9. A polypeptide that binds to the androgen receptor, said polypeptide comprising the amino acid sequence Ser-X-Ar-X-X-Ψ-Ar, wherein:

Ar is an aromatic amino acid;

X is any amino acid; and

Ψ is a hydrophobic amino acid.

30

10. The polypeptide of claim 9, wherein

Ar is selected from the group consisting of Tryptophan, Phenylalanine, and Tyrosine;

5 Ψ is selected from the group consisting of Valine, Leucine, Isoleucine, and Methionine;

β is selected from the group consisting of Lysine, Arginine, and Histidine; and

Δ is selected from the group consisting of aspartic acid and glutamic acid.

11. A polypeptide that binds to the androgen receptor, said polypeptide comprising a
10 sequence selected from the group consisting of:

SRWQALFDDGTDTSR (SEQ ID NO:5);

SSKFAALWDPPKLSR (SEQ ID NO:6);

SSAGGHGKNKWAGLWRESR (SEQ ID NO:7);

SSTGIQSKFQALFSR (SEQ ID NO:8);

15 SSKFYELFRNVNLSR (SEQ ID NO:21);

SSFFSDLFLLPEGRQAASR (SEQ ID NO:41);

SRFADIFREAQQESR (SEQ ID NO:22);

SRFSELFISPYVPDGLSR (SEQ ID NO:23);

SRFAELWEMDWGELVDPSR (SEQ ID NO:24);

20 SRWAEVWDDNSKVSER (SEQ ID NO:42);

SRWNDLFSLENTSARPNSR (SEQ ID NO:43);

SRWC DLWEGRVECMKEVASR (SEQ ID NO:44);

SSLGRQHWLELWGATDGSR (SEQ ID NO:45);

SRFADLFSERGSSR (SEQ ID NO:46);

25 SRFLDVFAADWEAGGGASR (SEQ ID NO:47);

SSEVTGMRFRDLFSR (SEQ ID NO:48);

SRFSDCYKMQSDCSR (SEQ ID NO:49);

SSSGRTSGAWERLWHGSR (SEQ ID NO:50);

SSMTPFEMLYINGHSRLSR (SEQ ID NO:51);

30 SSMGGATKWDVLWSR (SEQ ID NO:52);

SRTSQMGAFEWAFRGAESR (SEQ ID NO:53);

SRFDSLFGVWAGGEAMGSR (SEQ ID NO:29);

SRFEHLWKYELTDSR (SEQ ID NO:30);
SSRWESLWTGEANRSESR (SEQ ID NO:31);
SRFEALYLDRTGLHTDTSR (SEQ ID NO:32);
SSRFESLFAGEKESR (SEQ ID NO:54);
5 SSDSSRWELLWGRANGHSR (SEQ ID NO:55);
SRFEWMFNLDPGNSR (SEQ ID NO:56);
SSRFESMWPECHRSKSCSR (SEQ ID NO:57);
SRFGEMWPDRHSSSR (SEQ ID NO:58);
SSIGRLMFPDLEPSRSFSR (SEQ ID NO:59);
10 SRWMNMWDIADRSSR (SEQ ID NO:60);
SRGLWDFRAMFQEEHSSR (SEQ ID NO:61);
SRFTEMWLAAETESR (SEQ ID NO:62);
SRFQDMFDSFPSHEFSGSR (SEQ ID NO:63);
SSNSLFQTMYLQGANFSSR (SEQ ID NO:37);
15 SRFSAMFWANQADSR (SEQ ID NO:64);
SRFTDMFLEETGPSR (SEQ ID NO:65);
SSFTNMFGQYAPLHSFDSR (SEQ ID NO:66);
SSFERLWNNARLNSDSSR (SEQ ID NO:67);
SSFASVFFPPLADQLGNSR (SEQ ID NO:68);
20 SSFWEVWSGESARSR (SEQ ID NO:69);
SSFKNLWDAADGNSR (SEQ ID NO:70);
SSYWSTFWQSELNSR (SEQ ID NO:71);
SSCASFKGLWAGMGEMGSR (SEQ ID NO:72);
SSASFRSLFSASESR (SEQ ID NO:73);
25 SSWSSLFEYDFPSTVGLSR (SEQ ID NO:74);
SSEGQLFNRMFHHSR (SEQ ID NO:75);
SRLNCLFSTDGNSVCSR (SEQ ID NO:76);
SSSPFRSLFSNGDSR (SEQ ID NO:38);
SSPFYTLFAPFTTSPVSR (SEQ ID NO:39);
30 SSLFANRFFESVCVGGGLCSR (SEQ ID NO:40);
SSVSRGGWLGLWNNEKVSR (SEQ ID NO:77);
SSFQNFFAAPYMESPLSSR (SEQ ID NO:13);

SRFHSEWVPESWEAKVSR (SEQ ID NO:14);
SRFADFFRNEGLGSR (SEQ ID NO:15);
SSYNSSNGKFAKFWPTYSR (SEQ ID NO:16);
SSNTPRFKEYFMQSR (SEQ ID NO:78);
5 SRAGKFDRFWQEESR (SEQ ID NO:79);
SRFEAFYGVGEEGSVGASR (SEQ ID NO:80);
SSFDFKLYLDEYHGRGYQSR (SEQ ID NO:81); and
SRLSDLVKGAEQCVGCMGSR (SEQ ID NO:82).

- 10 12. Methods of analyzing the surface conformation of a protein using one or more of the polypeptide sequences in claims 1, 5, 7, or 9.
13. Methods of identifying modulators of protein function using one or more of the polypeptide sequences in claims 1, 5, 7, or 9.
- 15 14. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and one or more of the polypeptide sequences in claims 1, 5, 7, or 9.
15. A polypeptide that comprises at least 50% amino acid sequence identity to one or
20 more of the polypeptides of claim 11.
16. A peptide that binds to the androgen receptor, said binding being competitively inhibited by one or more of the polypeptides of claims 1, 5, 7, or 9.
- 25 17. A chimeric protein comprising a polypeptide according to any one of claims 1, 5, 7, or 9 and at least a portion of a filamentous phage protein, said portion of said filamentous phage protein being sufficient for integration of said chimeric protein into the coat of phage particles so as to display said polypeptide.
- 30 18. A filamentous phage displaying a polypeptide according to one or more of claims 1, 5, 7, or 9.

19. The polypeptide of any of claims 1, 5, 7, or 9 which is labeled with a detectable label.

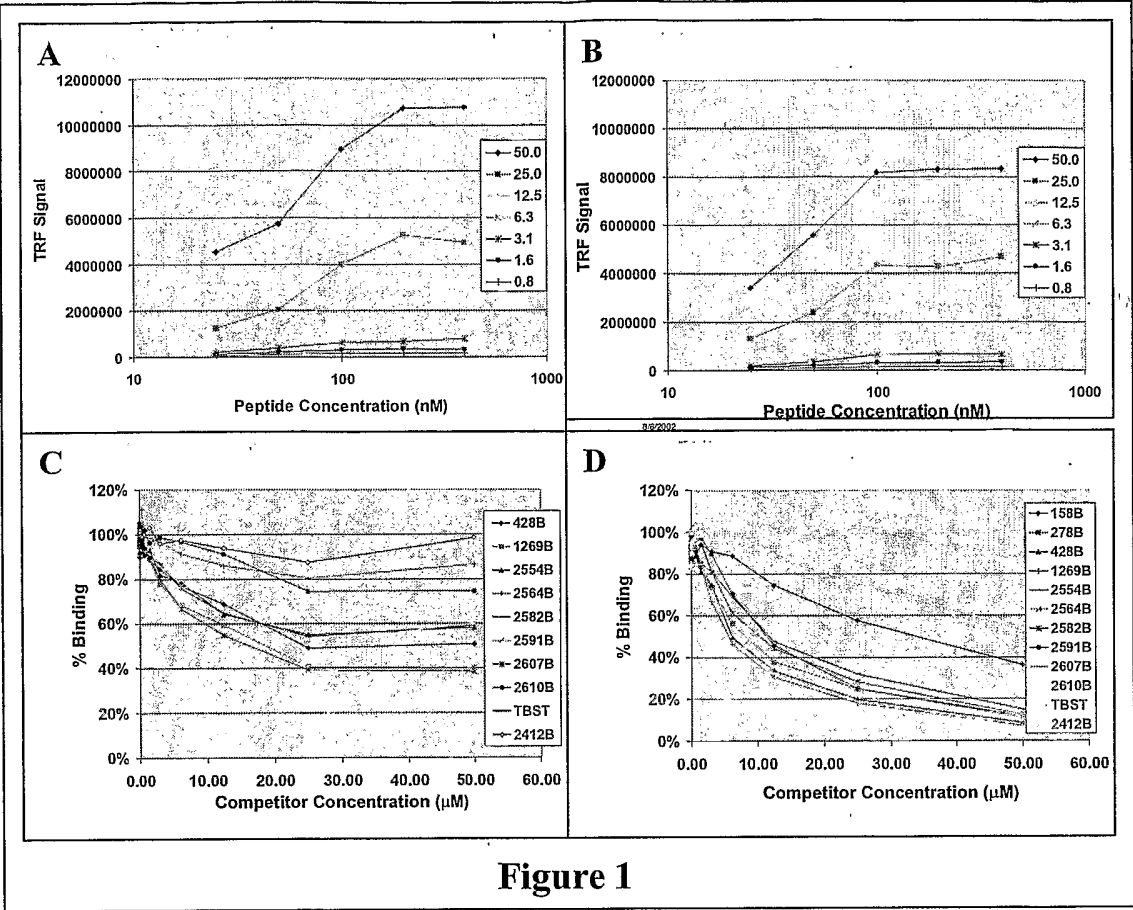
20. The polypeptide of claim 19, wherein said label is selected from the group
5 consisting of radiolabels, fluorophores, chromophores, enzymes, and combinations thereof.

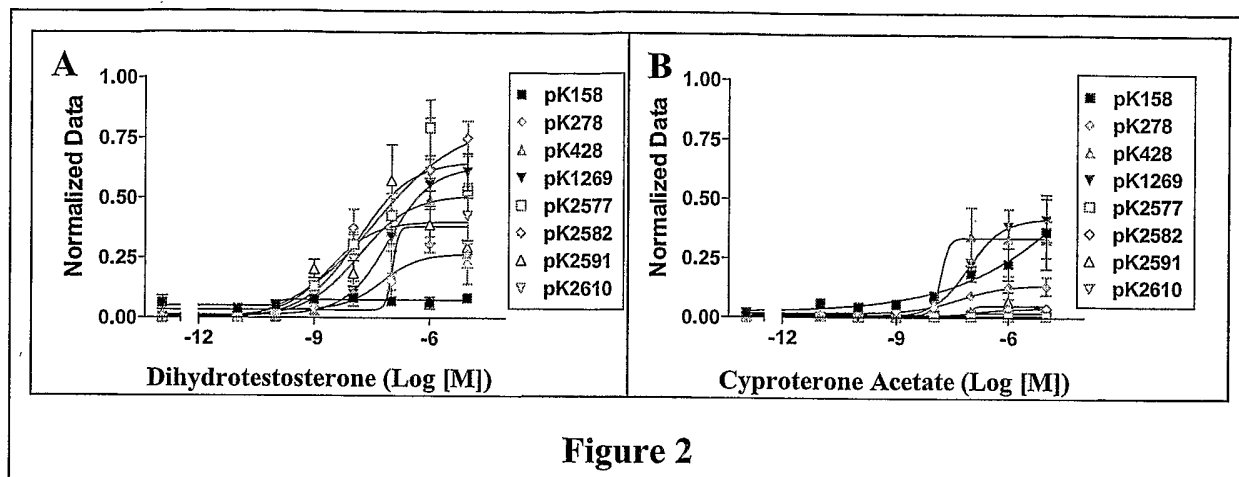
21. A method of diagnosing a disease in a patient characterized by abnormal levels of activation of androgen receptor (AR) comprising (1) providing a sample of body fluid or
10 tissue of said patient; (2) administering a diagnostically effective amount of the pharmaceutical composition of claim 14; and (3) assaying the amount of activated AR in said body fluid or tissue of said patient.

22. A method for treating a patient suffering from a disease characterized by abnormal
15 levels of activation of androgen receptor (AR), comprising administering to said patient a therapeutically effective amount of the pharmaceutical composition of claim 14.

23. The method of claim 21 or 22, wherein said disease is prostate cancer.

20





<110> Buehrer, Benjamin M.
Barnett, Thomas R.

<120> ANDROGEN RECEPTOR INTERACTING PEPTIDES

<130> 102421-101

<150> US 60/446,955

<151> 2003-02-12

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Ser Leu Phe Ala Asn Arg Phe

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1 5 10 15

Ser Ser Arg

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1 5 10 15

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Val Ser Arg

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Leu Cys Ser Arg

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Ser Arg

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<400> 58

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<400> 69

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<400> 75

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<211> 19

<212> PRT

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<211> 19

<212> PRT

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<210> 78

<211> 15

<212> PRT

<213> Mammal

<400> 78

Ser Ser Asn Thr Pro Arg Phe Lys Glu Tyr Phe Met Gln Ser Arg
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1 10 15
Ala Ser Arg

<210> 81
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Gln Ser Arg

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<211> 20
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<400> 82
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Met Gly Ser Arg
20