Estrogen receptor β and its domains interact with casein kinase 2, phosphokinase C and N-myristoylation sites of mitochondrial and nuclear proteins in mouse brain

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Running title: ERβ interacting mitochondrial and nuclear proteins in brain

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Keywords
Estrogen signaling; interacting proteins; coregulators; mitochondrial and nuclear proteins; CK2 and PKC phosphorylation site; N-myristoylation site; brain

Background: Estrogen receptor β and its domain interact with a host of brain mitochondrial and nuclear proteins.

Result: Estrogen receptor β interacting brain mitochondrial and nuclear proteins have consensus motifs.

Conclusion: Estrogen receptor β interacts with casein kinase 2, phosphokinase C and N-myristoylation sites present in mitochondrial and nuclear proteins.

Significance: This might be useful to regulate estrogen dependent gene regulation in brain for therapeutics.

SUMMARY
The localization of estrogen receptor (ER)β in mitochondria suggests ERβ dependent regulation of genes which is poorly understood. Here, we analyzed the ERβ interacting mitochondrial as well as nuclear proteins in mouse brain using pull down assay and matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS). In case of mitochondria, ERβ interacted with six proteins of 35kD-152kD, its transactivation domain (TAD) interacted with four proteins of 37kD-172kD, and ligand binding domain (LBD) interacted with six proteins of 37kD-161kD. On the other hand, in nuclei, ERβ interacted with seven proteins of 30kD-203kD, TAD with ten proteins of 31kD-160kD, and LBD with fourteen proteins of 42kD-179kD. For further identification, these proteins were cleaved by trypsin into peptides and analyzed by MALDI-MS using mascot search engine, immunoprecipitation, immunoblotting and far-western blotting. To find out the consensus binding motifs in interacting proteins, their unique tryptic peptides were analyzed by the motif scan software. All the interacting proteins were found to contain casein kinase (CK) 2, phospho kinase (PK) C phosphorylation and N-myristoylation sites. These were further confirmed by peptide pull down assay using specific mutation in the interacting sites. Thus the present findings provide evidence for the interaction of ERβ with specific mitochondrial and nuclear proteins through consensus CK2, PKC phosphorylation and N-myristoylation sites, and may represent an essential step towards designing selective...
ER modulators for regulating estrogen mediated signaling.

Introduction

Estrogen receptor (ER), a ligand-activated transcription factor belonging to the nuclear receptor superfamily, has two main types - ERα and ERβ. These receptors are localized intracellularly and following ligand binding, they regulate a large number of estrogen responsive genes (1, 2). They are also present in the plasma membrane and involved in many rapid, non-genomic effects via several signaling pathways (3, 4). In addition, ERβ is located in the mitochondria of estrogen target cells, including human breast cancer MCF-7 cells (5, 6). ER interacts with a large number of factors such as steroid receptor coactivator (SRC) -1 (7), glucocorticoid receptor interacting protein (GRIP)-1 (8), transcriptional intermediary factor (TIF)-2 (9) and others (10) which may activate (coactivators) or suppress (corepressors) the transcription of specific genes. Coactivators exhibit a region called helix-12 which includes ‘NR boxes’, each containing a LXXLL motif (where L is leucine and X represents any amino acid) (11), through which it interacts with ER as shown by functional in vitro and in vivo studies (12, 13). Mapping studies with corepressors reveal that the receptor interaction and repression functions are separately located towards the carboxy-terminal (14).

The corepressors namely silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT)and N-CoR have interaction motifs of short peptides comprising of 19 amino acids and 17 amino acids, respectively, with an internal signature motif (I/L)XX(I/V)l, which is sufficient for receptor interaction and ligand-induced dissociation. These motifs can adopt an amphipathic α-helical conformation, reminiscent of the signature motif LXXLL within coactivators (15). Mitochondrial localization of ERβ helps to understand the mechanism(s) of neuronal death and develop neuroprotective drugs and therapeutic strategies that can delay or prevent Alzheimer’s disease and other chronic neurodegenerative conditions. In the brain, estrogens exhibit multiple effects that enhance or preserve functions during pathologic circumstances such as excitotoxicity, oxidative stress and neurodegeneration. However, the ERβ and its domain interacting proteins and their cellular functions are poorly understood in the brain, specifically in mitochondria. Here we report the identification of ERβ, transactivation domain (TAD) and ligand binding domain (LBD) interacting proteins from the mitochondrial and nuclear extract of the mouse brain by pull down and matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS) followed by analysis with mascot search engine and motif search software to determine the consensus binding motifs.

Experimental procedures

Cloning and expression of ERβ, TAD and LBD

Detail procedures were discussed in Supplemental Materials and Methods. Briefly, same procedures were followed as reported earlier (16).

Purification of recombinant proteins

These were discussed in Supplemental Materials and Methods. Briefly, same procedures were followed as reported earlier (17).

Interaction of ERβ, TAD and LBD with mitochondrial and nuclear extract of mouse brain

Purification of mitochondria and preparation of mitochondrial extract - For mitochondrial purification (18), the mouse brain was dissected on ice and rapidly
minced and homogenized at 4°C in isolation buffer (225mM mannitol, 75mM sucrose, 5mM N-2-hydroxyethyl piperazine-N’-2-ethanesulhonic acid (HEPES), 1mM ethylene glycol-bis (2-aminoethyl ether) (EGTA), 1mg/ml BSA, pH 7.4). Then the homogenate was centrifuged at 600xg for 5min at 4°C. The supernatant was collected and centrifuged at 9,000xg for 8min at 4°C. Thereafter the pellet was resuspended in isolation buffer containing heparin to release the mitochondria from synaptosome and again centrifuged at 9,000xg for 8min at 4°C. Then the brownish mitochondrial pellet was resuspended in isolation buffer and spun at 9,000xg for 10min at 4°C. The pellet containing mitochondria was resuspended in suspension medium (isolation buffer with 0.02% w/v heparin). Before preparing extract mitochondria were observed under microscope to avoid contamination (Fig. 1). The suspension was incubated with lysis buffer containing 20mM HEPES (pH 7.6), 25% glycerol, 420mM NaCl, 1.5mM MgCl₂, 0.2mM ethylenediaminetetraacetic acid (EDTA), 0.5mM PMSF, 0.5mM DTT, 0.4mg/ml complete EDTA-free protease inhibitor (Roche Diagnostics, Germany) and stirred for 30min on ice. Then it was centrifuged at 25,000xg for 40min at 4°C. The supernatant containing the mitochondrial extract was checked quantitatively by Bradford method and qualitatively by silver staining. The purity of mitochondrial extract was checked by in gel assay of MnSOD and immunoblotting using β-actin and GAPDH antibody. The detailed procedure is given in SI Materials and Methods.

**MALDI-MS analysis**
The detailed procedure is given in SI Materials and Methods.

**Identification of putative proteins**
The detailed procedure is given in SI Materials and Methods.

**Identification of motifs**
The detailed procedure is given in SI Materials and Methods. Briefly, same procedure was followed as earlier reported (20).

**Peptide pull down assay**
All the peptide sequences were purchased from GL Biochem Ltd, Shanghai, China. The peptide sequence from N to C terminus; namely for (casein kinase) CK2-RRREDEESDDEEA, mutated CK2-RRREDEEPALDEEA, (phospho kinase) PKC- CRFARKGSLRQKNV, mutated PKC- CRFARKGPPAQKNV, N-myristoylation- RCRMGGFLATSLSW, and mutated N-myristoylation-RCRMAAIPLASLSW were used for the peptide pull down assay. All the peptide sequences were biotin tagged at N terminus. For the interaction study, CK2, PKC and N-myristoylation sites and their respective mutants were used. Briefly, one mg of each peptide was dissolved in one ml and aliquotted. Avidin beads were purchased from Sigma (USA), suspended in 400µl 1xPBS and incubated with 100µg of biotinylated peptide overnight. Then it was washed three times with 1ml PBS to remove unbound peptides. Thereafter 15µl of washed peptide bound beads and 5µg of purified ERβ, TAD and LBD were incubated in 400µl binding buffer for 4h at 4°C. After incubation, the beads were washed with washing buffer three times.
Then the beads-protein complex was resuspended in 40µl Laemmli buffer and boiled at 100°C for 5 min. Finally, it was centrifuged at 10,000xg for 5 min and supernatant was resolved by 7.5% SDS-PAGE and the band was detected by silver staining.

**Results and discussion**

Before conducting experiments using mitochondrial and nuclear fraction, purity of these fractions was checked. Presence of MnSOD activity in gel assay in the mitochondrial fraction and absence of GAPDH and β-actin by immunoblotting confirmed the purity of mitochondrial fraction (Fig. 1). Further, absence of MnSOD activity by *in* gel assay and presence of β-actin and GAPDH by immunoblotting confirmed nuclear extract purity (Fig. 1).

Before performing pull down assay, we have analyzed the ERβ and its domains interacting proteins using bioinformatics tools (STRING 8.0, PIP, BIND and DIP) and literature based studies (iHOP, NCBI and ISI web of knowledge). This showed several interacting partners of ERβ, but the pull down assay revealed relatively lower number of proteins, 16 in the range of 30kD-172kD in mitochondria (Fig. 2) and 31 in the range of 31kD-203kD in nuclei (Fig. 3). Surprisingly Timm44, a bona fide mitochondrial protein, was found in the nuclear fraction of mouse brain, whereas the nuclear pore complex 86kD protein was found in the mitochondrial fraction. Timm44 crosses through the nuclear pore and attaches to the translocator proteins of nuclear membrane (21). Similarly, Selvam et al (22) identified some of the nuclear pore complex binding proteins in the mitochondrial extract of rat kidney. Two steps were used for identifying the proteins using mascot search engines. In the first step, all the peptide peaks including intense ions 842.933, 1476.33 1941 and 2384.7 in Fig 1A, 1123.9 in Fig 1C and 1123.9 in Fig ID were used. It provided a list of proteins with various mascot scores and best matched unique peptide sequences. In the second step, these unique sequences were used to make identification more stringent with higher mascot score. Detailed procedure has been provided in supplementary materials and methods section. Also, one excel file showing procedure has been provided in the supplementary data. Thus, the proteins identified were more stringent with higher mascot score and collated to brain functions.

**Interaction with mitochondrial proteins**

**ERβ interacting proteins**

ERβ showed interaction with six proteins of 152kD (prenylated protein tyrosine phosphatase, peaks-36, unique peptides-11 and mascot score-149), 101kD (BC026557 similar to nucleoporin, peaks-29, unique peptides-11 and mascot score-153), 86kD (AK009187 similar to mitotic phosphoprotein, peaks-35, unique peptides-13 and mascot score-170), 71kD (phosphoinositol 3-phosphate-binding protein, peaks-26, unique peptides-22 and mascot score-190), 40kD (MKIAA1251 protein peaks-31, unique peptides-17 and mascot score-84), and 35kD (MUSACMHCA alpha cardiac myosin heavy chain peaks-31, unique peptides-14 and mascot score-56) (Fig. 2A, Table 1A, Supplemental Fig. 1A-F). The 152kD protein matches to a receptor-like transmembrane protein and causes dynamic tyrosine phosphorylation and dephosphorylation at serine/threonine and participates in many aspects of cellular, physiological and pathological processes (23). The 86kD protein is a member of nucleoporins which constitute the nuclear pore complex and interacts with transiently associated nuclear transport factors (24). The 71kD protein recruits cytosolic proteins...
to specific membrane compartment and acts as a spatial regulator of many cellular processes including endocytic trafficking and membrane fusion (25). The 40kD protein is involved in G-protein signaling and vesicle trafficking. Its mRNA in mouse brain has long 3'-UTR which increases with evolutionary age and organism complexity (26).

**TAD interacting proteins**

TAD showed interaction with four proteins of 172kD (Abhd4 protein, peaks-32, unique peptides-13 and mascot score-160), 58kD (aldehyde reductase, peaks-29, unique peptides-17 and mascot score-199), 50kD (survival of motor neuron, peaks-31, unique peptides-4 and mascot score-42) and 43kD (AIG1 family/TONB Box N terminus, peaks-36, unique peptides-20 and mascot score-158) (Fig. 2B, Table 1B, Supplemental Fig. 1G-J). The 172kD protein is a lysophospholipase selective for N-acyl phosphatidylethanolamine (NAPE) and is involved in the biosynthesis of endocannabinoid anandamide by hydrolyzing the sn-1 and sn-2 acyl chains from NAPE generating glycerophospho-N-acyl ethanolamine (GP-NAE), an intermediate for N-acyl ethanolamine biosynthesis. It hydrolyzes substrates bearing saturated, monounsaturated and polyunsaturated N-acyl chains. Its interaction with ERβ suggests the role of estrogen in the regulation of the cannabinoid receptor and neuroprotection against cannabinoids (27). The 58kD protein is aldo-keto reductase which catalyzes a number of redox transformations of glucose, steroids, glycosylation end products, lipid peroxidation products and environmental pollutants involved in biosynthesis, intermediary metabolism and detoxification. It plays an important role in the detoxification of a large number of pharmaceuticals, drugs and xenobiotics (28).

The 50kD protein binds to small actin-binding protein and is involved in snRNP transport and nuclear RNA splicing (29).

**LBD interacting proteins**

LBD interacted with six proteins of 161kD (E130112J05 product protein, peaks-36, unique peptides-7 and mascot score-126), 103kD (Arhgap28 protein, peaks-33, unique peptides-24 and mascot score-188), 88kD (AK014612, f-box only protein, peaks-33, unique peptides-15 and mascot score-162), 73kD (LOC433520 protein, peaks-32, unique peptides-9 and mascot score-92), 41kD (kinesin-related microtubule-based motor protein, peaks-32, unique peptides-13 and mascot score-62) and 37kD (vinculin, peaks-32, unique peptides-20 and mascot score-136) (Fig. 2C, Table 1C, Supplemental Fig. 1K-P). The 103kD protein is cytoskeletal and provides mechanical support to the plasma membrane and is involved in cell signaling, protein trafficking, control of cell proliferation, cell migration, adhesion, cytokinesis and transcriptional activity (30). The 41kD is putatively identified as kinesins which are expressed in juvenile tissues including differentiated young neurons. Its expression is decreased considerably in adult mouse. It is involved in the transport of organelles through the axon, nucleotide-dependent binding to microtubules, microtubule-activated ATPase activity and microtubule plus-end-directed motility (31). The 37kD vinculin is a member of kinesin-like proteins (KLPs). It is involved in the localization of the mitotic spindle and ATP hydrolysis to move along microtubules in a unidirectional manner, transporting chromosomes or other microtubules towards either the plus or minus end of the microtubule polymer (32).
Interaction with nuclear proteins

ERβ interacting proteins

ERβ interacted with seven putative proteins of 203kD, 140kD, 84kD, 43kD, 39kD, 33kD and 30kD in mouse brain (Fig. 3A, Table 2A, Supplemental Fig. 2A-F). The 140kD protein was identified as estrogen receptor associated protein (ERAP) 140 by immunoblotting, immunoprecipitation and far-western blot analysis (Fig. 4A), and the remaining proteins by MALDI MS. The variation in MW determined by pull down and MALDI-MS analysis was due to their multisubunit characteristics and post-translational modifications, especially glycosylation. The 203kD protein matches putatively to MKIAA1251 (peaks-20, unique peptides-22 and mascot score-109) and Ugcgl2 (peaks-20, unique peptides-13 and mascot score-104). The 140kD protein is characterized as a conserved tissue specific nuclear receptor coactivator with abundant expression in brain, exclusively in neurons. Its homologues are present in both invertebrates as well as vertebrates including human (33).

We recently reported that mouse ERβ interacts with estrogen receptor associated protein (ERAP) 140 and shows variation in the levels of interaction and expression during aging of mouse brain (19). The 84kD protein matches to MMU10551 (peaks-28, unique peptides-16 and mascot score-84), which is a member of the Ras superfamily and plays an important role in the regulation of L-type calcium channels and diverse cellular processes like cell growth, differentiation, cytoskeleton regulation, vesicular transport, cytokinesis, phagocytosis, pinocytosis, cell migration, morphogenesis and axon guidance (34). The 43kD band matches to salivary androgen-binding protein (ABP) gamma subunit fragment (peaks-25, unique peptides-7 and mascot score-63), which plays a role in sexual selection during brain development (35). The 39kD protein matches to adult male epididymis cDNA, RIKEN full-length enriched library, clone: 9230110G02 product: ATP/GTP-binding site motif A (P-loop)/Zinc finger (peaks-25, unique peptides-19 and mascot score-183) (36). The 33kD protein matches to MMFXR1H9 (peaks-29, unique peptides-17 and mascot score-175), which is present in the brain and shows estrogen dependent expression and regulation (37). The 30kD protein matches to adult male small intestine cDNA clone product, RIKEN full-length enriched library, clone:2010015J01 product:weakly similar to Arp2/3 complex 16kD subunit (P16-ARC, actin-related protein 2/3 complex subunit 5, peaks-18, unique peptides-12 and mascot score-156) which is present in the brain of fetal down syndrome patient and is responsible for neuronal and glial migration during brain development (38).

TAD interacting proteins

TAD interacted with ten proteins of 160kD, 100kD, 98kD, 85kD, 69kD, 61kD, 53kD, 45kD, 34kD and 31kD (Fig. 3B, Table 2B, and Supplemental Fig. 2G-O). The 160kD protein was identified as amplified in breast cancer (AIB) 1 by immunoblotting, immunoprecipitation and far-western blot analysis (Fig. 4B). AIB1 is a member of the nuclear coactivator (NCoA-3) and p160 steroid receptor co-activator (SRC) family, which includes SRC-1 and TIF-2 and plays a role in breast cancer and enhances angiogenesis and neurogenesis (39). We recently reported that mouse ERβ interacts with amplified in breast cancer (AIB) 1 (38) and showed variation in the levels of interaction and expression during aging of mouse brain. The 100kD protein is probably an iron binding protein P97 (peaks-31, unique peptides-8 and mascot score-127), but its physiological role is not clear in brain. Originally, it was discovered in melanoma cells, but now it is known to be...
expressed at varying levels in several kinds of tumors. In normal tissues, it is expressed at high levels in fetal gut, adult sweat gland and probably brain capillaries. P97 is structurally homologous to transferrin and is shown to bind to iron and zinc. It is reported as a marker in Alzheimer patients, which show its elevated level in serum (40). The 98kD protein is identified as an adult inner ear protein (peaks-20, unique peptides-6 and mascot score-113), which is a member of Nkx5-1 family and has various roles in inner ear and hindbrain development (42). The 85kD protein matches to MKIAA 0670 (peaks-24, unique peptides-23 and mascot score-139), which is a member of KIAA family with unknown biological function (43). The 69kD protein has been identified as 18th day whole body protein (peaks-26, unique peptides-9 and mascot score-112), and acts as a homeodomain transcription factor in brain. The 61kD protein is a ankyrin protein (peaks-31, unique peptides-19 and mascot score-187), expressed in brain and plays a vital role in energy homeostasis (44). The 53kD protein is identified as bone marrow macrophage tRNA synthetase (peaks-35, unique peptides-9 and mascot score-112), and functions as a cofactor in metabolic activity (46). The 45kD protein is heparan sulphate sulphotransferase isoform 6 (peaks-29, unique peptides-16 and mascot score-182) and is responsible for the transportation of proteins to membrane and sub-organelles including mitochondria. It controls the oxidative phosphorylation and takes part in the respiratory chain enzyme kinetics (48). The 99kD protein is identified as an adult bone marrow protein, (peaks-22, unique peptides-13 and mascot score-139) and plays an important role in cell proliferation during development.

**LBD interacting proteins**

LBD interacted with 14 proteins of 179kD, 140kD, 138kD, 136kD, 115kD, 99kD, 88kD, 69kD, 60kD, 58kD, 49kD, 45kD, 43kD and 42kD (Fig. 3C, Table 2C and Supplemental Fig. 2P-X). Among them 140kD, 138kD, 60kD, 45kD and 43kD were identified as ERAP 140, TrkA, Src, pCREB and cAMP response element binding protein (CREB), respectively, by immunoblotting, immunoprecipitation and far-western blot analysis (Fig. 4C).

The 179kD protein has been identified as a molydopterin converting factor subunit 2 containing protein (peaks-24, unique peptides-12 and mascot score-163), and functions as a cofactor in metabolic activity (46). The 138kD protein is Trk A, which is known for the autophosphorylation and recruitment of signaling molecules and activates a receptor-specific intracellular signaling pathway that relays information to nucleus and other intracellular compartments (47). The 136kD protein is identified as 2nd day oviduct protein (peaks-24, unique peptides-12 and mascot score-122), and has no well known function. The 115kD protein is Tim 44 (peaks-18, unique peptides-15 and mascot score-137), which is responsible for the transportation of proteins to membrane and sub-organelles including mitochondria. It controls the oxidative phosphorylation and takes part in the respiratory chain enzyme kinetics (48). The 99kD protein is heparan sulphate sulphotransferase isoform 6 (peaks-29, unique peptides-16 and mascot score-182), and is reported in the brain. The 88kD protein matches to tomoregulin (peaks-16, unique peptides-13 and mascot score-140), and is a transmembrane protein predominantly expressed in the brain. The 69kD protein has been identified as
senataxin (peaks-23, unique peptides-21 and mascot score-70), which causes a rare juvenile-onset form of amyotrophic lateral sclerosis (ALS). The 58kD matches to SRY protein (peaks-24, unique peptides-14 and mascot score-127), known as the key sex-determining protein that directs gonads to develop as testes rather than ovaries. The 60kD protein is identified as Src which belongs to a family of highly homologous proteins with protein tyrosine kinase (PTK) activity and resides in the cytoplasm and perinuclear space. Recent reports suggest that Src is also located at the plasma membrane and several subcellular compartments including the nucleus (49) and involves in signal transduction. The 49kD protein has been identified as a member of KIAA gene family (peaks-23, unique peptides-13 and mascot score-118), and its role in brain function is not clear (41). The 45kD and 43kD proteins have been identified as pCREB and CREB, respectively. They are well known transcription factors involved in multiple brain functions. Moreover, 42kD protein is identified as alanine region protein (peaks-29, unique peptides-9 and mascot score-123), and is known for its role in signal transduction in the brain (50).

Identification of consensus motifs
As all the mitochondrial and nuclear proteins interacted with ERβ and its domains, we assume that these proteins have a common consensus binding motifs. Therefore, all the interacting proteins were analyzed by motif scan, and interestingly they were found to contain consensus motifs CK2, PKC phosphorylation and N-myristoylation sites (Fig. 5 and Fig. 6). This was also confirmed by peptide pull down assay using specific biotinylated CK2, PKC and N-myristoylation peptides. CK2, PKC phosphorylation and N-myristoylation sites showed interaction with ERβ, TAD and LBD whereas no interaction was observed with their respective mutants (Fig. 7). The gels show the binding difference between normal and mutated peptides with ERβ and its domains. In string 8.0, peptide sequences did not show any match with putative proteins. So it is likely that these are bacterial proteins contaminations. Both CK2 and PKC phosphorylation sites start with serine or threonine residue, and phosphorylation at these sites is responsible for the interaction of all the putatively identified proteins with ERβ resulting in the downstream functions, namely neuronal architecture, migration of neuronal and glial cells during development and cell proliferation (51). Further, N-myristoylation is involved in the anchoring of proteins and their targeting to cell membrane and plays a critical role in cell signaling, apoptosis and extracellular protein export. The myristoyl-CoA and N-myristoyltransferase (NMT) recognize the sequence motif of myristate site at N-terminal glycine residues. Apart from anchoring, myristoylation is also involved in the docking of Ca²⁺ (52).

As protein interactions are transient, our proteomic approach precisely analyzes the interacting proteins by pull down assay followed by MALDI-MS and identifies their motifs by motif scan software. The identification of ERβ interacting proteins in brain mitochondria indicates the importance of ERβ in the regulation of mitochondrial gene expression. This is contradictory to an earlier observation in which ERβ was reported as a poor transcription factor (53, 54). The present study is of great significance as it advances our knowledge of nuclear receptor and its interaction and functional analysis in the brain mitochondria. The putatively identified proteins are involved in cellular signaling in mitochondria. The reduced level of putatively identified survival motor neuron (SMN) protein causes spinal muscular...
atrophy. The CK2, PKC and N-myristoylation sites have been identified for the first time as consensus motifs in all ERβ interacting proteins in the brain mitochondria. This raises the possibility to regulate the estrogen dependent gene expression through ERβ in health and disease (10).

Taken together, our studies showed that ERβ and its domains interact with the consensus CK2, PKC phosphorylation and N-myristoylation sites of specific mitochondrial as well as nuclear proteins. Evidences from literature suggest that these interacting proteins are involved in neuronal architecture, cytoskeleton integrity, signal transduction and cell proliferation. The identification of consensus binding motifs in ERβ interacting proteins of the brain mitochondria represents an essential step towards designing selective ER modulators for understanding estrogen mediated signaling.

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Figure legends
Fig.1. Photograph observed under microscope showing A) mitochondria B) nuclei.
C) In gel assay showing presence of MnSoD in lysate and mitochondrial fraction and absence in nuclear fraction. D) Immunoblotting showing presence of β-actin in lysate and nuclear fraction and absence in mitochondrial fraction. E) Immunoblotting showing presence of GAPDH in nuclear fraction and absence in mitochondrial fraction.

Fig.2. Pull down assay showing interaction of (A) ERβ, (B) TAD and (C) LBD with mitochondrial proteins of the mouse brain. Interacting prey proteins were resolved by SDS-PAGE, and detected by silver staining. Lane M: Marker (Fermentas); lane 1: Mitochondrial extract; lane 2: beads; lane 3: Interacting proteins in the presence of estradiol; lane 4: Interacting proteins in the absence of estradiol.

Fig.3. Pull down assay showing interaction of (A) ERβ, (B) TAD and (C) LBD with nuclear proteins of mouse brain. Upper panel: Interacting prey proteins were resolved by SDS-PAGE, and detected by silver staining. Lane M: Marker (Fermentas); lane 1: Nuclear extract; lane 2: beads; lane 3: Eluate following interaction of his-tag protein with nuclear extract; lane 4: Interacting proteins in the absence of estradiol; lane 5: Interacting proteins in the presence of estradiol. Lower panel: Immunoblot analysis showing interacting proteins (present in lane 5) as ERAP 140, AIB 1, Trk A, Src, pCREB and CREB.
Fig.4. Identification of (A) ERβ, (B) TAD and (C) LBD interacting proteins. *Upper panel* Immunoprecipitation shows the interaction of ERAP 140 with ERβ, AIB1 with TAD and Trk A, Src, pCREB and CREB with LBD. Briefly, nuclear extract from mouse brain was immunoprecipitated with anti-ERβ antibody, followed by immunoblotting with anti-ERAP 140, anti-AIB1, anti-Trk A, anti-Src, anti-pCREB and CREB.

*Middle panel:* In vitro labeling of fusion proteins and their purification. Lane1: $^{35}$S methionine labeled ERβ with reaction mixture; lane 2: purified labeled ERβ, lane 3: γ-labeled $^{32}$P TAD reaction mixture; lane 4: purified labeled TAD; lane 5: γ-labeled $^{32}$P LBD with reaction mixture; lane 6: purified labeled LBD.

*Lower panel:* Identification of interacting proteins by far-western blotting. Lane 1: bacterial extract (negative control) and lane 2: mouse brain nuclear extract (positive control) were resolved by SDS-PAGE and transferred onto membrane. Subsequently the blot was denatured/renatured and hybridized with (A) $^{35}$S methionine labeled ERβ, (B) γ-labeled $^{32}$P TAD and (C) γ-labeled $^{32}$P LBD, and signals were detected by autoradiography.

Fig.5. Aligned unique peptides of mitochondrial proteins showing CK2 (red, four in number and starts with S/T), PKC (green, three in number and starts with S/T) and myristoylation sites (blue, five in number and starts with G) represented by bold amino acids interacting with (A) ERβ, (B) TAD and (C) LBD.

Fig.6. Aligned unique peptides of nuclear proteins showing CK2 (red, four in number and starts with S/T), PKC (green, three in number and starts with S/T) and myristoylation sites (blue, five in number and starts with G) represented by bold amino acids interacting with (A) ERβ, (B) TAD and (C) LBD.

Fig.7. Peptide pull down assay showing interaction of (*upper panel*) ERβ, (*middle panel*) TAD and (*lower panel*) LBD with peptides containing CK2 (A), PKC (B) and N-myristoylation sites (C) and their respective mutations. The pulled down proteins were resolved by SDS-PAGE, and detected by silver staining. Lane M: Marker (Fermentas); lane 1: beads; lane 2: wash before binding of proteins (WBP); lane 3: wash after binding of proteins (WAP); lane 4: eluate showing proteins interacting with normal peptides (ENP); lane 5: eluate showing proteins interacting with mutated peptides (EMP).
### Fig. 3

| kD | M  | 1  | 3  | 4  | 5  |
|----|----|----|----|----|----|
| 140kD | ERAP | 140 |     |     |     |
| 160kD | AIB1 |     |     |     |     |

| kD | M  | 1  | 2  | 3  | 4  | 5  |
|----|----|----|----|----|----|----|
| 140  | ERAP | 140 |     |     |     |     |
| 138  | Trk A |     |     |     |     |     |
| 60   | Src |     |     |     |     |     |
| 45   | pCREB |     |     |     |     |     |
| 43   | CREB |     |     |     |     |     |
|   | A |   | B | C |
|---|---|---|---|---|
|   | ERβ | IgG | ERβ | IgG | pCREB | CREB |
|   | AIB1 |   | Trk A |   |   |   |
|   |   |   | Src |   |   |   |
|   | ERAP 140 | ERβ IgG | ERβ IgG | ERβ IgG | pCREB | CREB |
|   |   |   |   |   |   |   |
| 1  | 2 | 3  | 4  | 5  | 6  |
|   |   | 1  | 2  |   |   |
|   |   |   |   |   |   |
|   |   |   |   |   |   |

Fig. 4.
Fig. 5A

152kD (Myristyl:1)

1  RGAFNASKQYRPMKRMPYMRLQDLLLYEKCYYGTVTVLTVVRYYEDAVQFRFDRTNGHCCV
61  QAPVIVALALEICGKMYNRMARPVEYSENAMRFEEPGCCVAVHVCAVAGRAPPVALALI
121  EGCMMKYGIVHDWPFDGAPPNQIVDDWLNLLKT 156

101kD (CK2:3, Myristyl:2)

1  GMSEALKVGMSEALKVVGVVGHFSTCQVQTULCIGVQGTSTLTYKDCVQWPGGSRAPPER
61  TVELQKASQKLASWQQGKMQVMAKSQKLASWQQGKMQVMAK 136
121  SWSMQMVQGSTLTYKD 136

86kD (CK2:2, PKC:6, Myristyl:1)

1  DESSLRSRAAASDQYQVISDRTQVSTMRPLATAYKASISGCPVVMEMRSGVLSSPLAFTTP
61  IRTVHMSNTRGNWMMHRIYSPPLLAGSSPPQQVVPAMKDAASTSDYQVISDRTQTPPKSPLLAGG
121  SPPQPVVPAMKDAASTSDYQVISDRTQTPPKSPLLAGG 181  FAVDQPAPTQILSEPMLGSPSTPK TQANISLLQSPVGLTPVPGQSMFPSANIPRKT 240

71kD (CK2:5, Myristyl:5)

1  TLPQNSKTGHCQLSPPAITTDNASTKEQSLQDECRGAWREYKDEISKEEKRGSHFPVG
61  VPLRTSEGPDYRLYKSMVEILRTERPSIQDVMEGSLKHKQVLRPEVQNHQKNMLDAAL
121  VQTPQEVGGSHPNAGPLATEADRVQDIQPRDDQPRCPKTMQCIDNKMENSDRGGVSPLLSFLAPTQKTA 181

40kD (CK2:5, PKC:2)

1  TRVAQKEELLEEEKVVYSDLARRTSQIFLHAKQDTCHKKEULKLEQVSEAIKTAATELAENKR
61  KSKAAATELAKERKSEDQVEQTEQATVFESQKQKPGKDIEGFEQNTKLEQYEVQLERTR
121  VELEAAEVTSAQLQETEKSELEAEVTSAQLQETEKSELEAATQISSDAQTFMDLRTRYHQQG
181  ASRQELPAQLQNQSDLKIDALDLDVWAVSGSSEEEPRKPAQSLPGMEQFSACLEKQ 235

35kD (CK2:5, PKC:2, Myristyl:4)

1  LRSDSLRSHEDCDLLERQREQLEEGKALQTENGELAQEALISQLTRQGKLKANLALAHQSS
61  RHIEDEQALALLYKQKKLLEEGATSVQIEMNKKRLEDDVTSNMEQIIKAKADLEELATLQ
121  HEATAAALRKKNALAHQSSMRHCDLLEKHADSVAELEGQIDNLRQVLEDLKLITQES
181  IMDLENDKLSFKEKLDDVTSNMEQIIKAKADLEELATLQ 209

Fig. 5B

172kD (CK2:1, PKC:1, Myristyl:2)

1  IHHIRKDFRPFDRFKSRNPALVRLWDAVGFGRSMQRPDSYVRDAMMESFGWARRAMMESFG
61  WARRVKMQROPSYVRDAPPTWVKAVASVLGRSAMSEFGWARRPMLERDVTPMYGANT
121  WIDTSTGKKEVTNGIMPTMLLIGHSLGFLATSEIKYETMGIPTMILLGSHLGGFLATSE
181  SIKYPERV 189

58kD (CK2:1, Myristyl:4)

1  WQVQKHKGRSPARIALEVLAVKGAHLSAGYRHSPARILRLWEEFLVTSKLESVGSKAVYP
61  REMPLIGLGLTWKSYVYPMITVGDRLVEVLAVGLVYAYVPMITVGDRLVRHHPEDVEPA
121  LRTGGLVKALGLSSSNQRMSWRYIVIPMTVGDRLWNKDPHDVPEALRKHPDEPVLE
181  EPVVLALAEKHAWRHDEPDPEVLEEPVVLALAEKH 214

50kD (CK2:2, PKC:2, Myristyl:4)

1  QEQTSPHRQQEQTSPHRAMGSGGAGSEQEDTVLFRRMAMGSGGAGSEQEDTVLFRRMAMGSG
61  GAGSEQEDTVLFRRMAMGSGGAGSEQEDTVLFRRMAMGSG 96

43kD (CK2:3, PKC:5, Myristyl:1)

1  IGPKAYRSAAGNSIGQKQAYSIFNYAFTIVALFTRKFSTVLFRTHMIVVFREETIKNI
61  GRSTETIIEGWKGILLLLGKQGAGKSVTAKAFASHRVSFDHMVTDRCQSESVSVRGKQA
121  FASHSRVWQGKKAHYRFMDTVEFGERHYAIYFNYAEDFDQALDKVIFIMEQPTQIPDPC
181  DPDPELRVFSFDHMVTDRCQSESVSVRQGDPPTGDPCDPDELRVLLMKGRLDESAYKN
241  HTFPQPHALLVTLLLLSLSK 261
**Fig.5C**

**161kD (PKC:3, Myristyl:6)**

1. RPGTEQGQSEARVRGQWGPQGSAACRGFFRLTTLPGWKTGQWGPSQAARGFLLVCSSG
2. RRLDELSLSFCAPSVAAGGCAAGTFVGFLVCCSSGRRPGTEQGQSEARVLDELSLSFCAPS
3. VAGGCAATFRVRL 134

**103kD (CK2:7, Myristyl:3)**

1. QLPSMKKEIESIKEQLPSMKKEIESIKE
2. EAAMLMKASQPGAGEPRCASRAVEESESGLESEGIFRLMLSNESLHPPSFVRSFGLAETGD
3. SVEDMKKIMSDPVPEGVIRHAPLKSJVSNSQASVSADSMEMEFLEINRMLSNESLHPPS
4. FSRSVFQALHLMVMAIPDNARMDVEWDSGGVVLTAYHSHARSMDTQAQALMAFFNKVIANE
5. SKNMNLWINSTVMAPPLFRRSRSKHTNGMSADAEQSVQSTLSDDDYYHKGKNIHLSSLIELTA
6. FDFTQGQLKRDFPVQGQPDSCEHATQLDGTKEGPDVQFQALHLMVMAIPDNARDG
7. PDIVQFQALHLMVMAIPDNARDG 383

**88kD (CK2:2, PKC:1, Myristyl:2)**

1. CYPREFNYYVRIRFLDEKNSFMNILEKVLQYHFSERQWYYHVHGKSTKETYKQFHQEK
2. WREQYGTQLCKHNFMIIEKVLKVSVLGNNIMWVYRMENLFSLNYDAAKKELLQ
3. TLYTSCLTVQYRGLTITDLPVCQLNIMQRFLDEKSGSFVSDSSYCNKESQLTSG
4. IAQKNFMNILEKVEN

**73kD (CK2:5, PKC:3)**

1. EKGPPRRRETLCRTYTVNVTMTVTNVVRTTTLEQGMASTKYSITEHIQERNVNNVVKPLH
2. KAYSEHIQRNNAISENITEHIQERDTVVTNTVQRTLEQGMSKYNPMNVTNVEKPLQEGV
3. TSKNPMPNLTNVKPLQEGVTFKEDNMNVTSMVPLQEAASVNAIKDNPMNVTTVKPL
4. HKAALAEISEHIQERNN

**41kD (CK2:2, PKC:4, Myristyl:5)**

1. ENLGNSRTVGREDAERRADSTGAKGTLAASVM.contacts.Eishments.RHISVTLWLLRELSDLVLAGSER
2. AEDGQTLGVFSKKEALLAEMGAVAMRECNASEDPNKLVRCHPLGPPVEDLSSLETEL
3. KIAELNTEWEEKLAVTSYNDQDLMDSGNKATAMVAALSPADINYETLSTLRYADRA

**37kD (CK2:3, PKC:2, Myristyl:3)**

1. LGTAAKAVIFTSKNGLVAEGHRILJLGAVAKVREGEGESQARATISPMVMADAAANFE
2. NHSGRLSTVEGIQAVSKYDTRENESDEPKFSLDASEAAIKDAVAGNISPSDQLQKSSTVE
3. GIQASVKTARELGTAALKAAAVGATANSLANNMMPYQRDDLAKCAASDELSKTSIPMVM
4. DAKAGLVVEGHRNAMMPYQRLLLPPGNNQQAYEHFTMKNCVKVANINQPOQMLVAGA
5. TSIARRVQDQLAQALDAARGEGESQPARAMTGLVDEAIDTKSLLDASEAAIKK 294
Fig. 6A

203kD (CK2: 7, PKC: 2)
1 SALLQKA <b>SEAE</b> LKQTRVQA QKEHQGAGSRQKY <b>SE</b>TEILAKENP DHVLKSYISDALRR
61 QQDTCHK KNLQD <b>SIKR</b> KRYISDARRHLGHKL <b>SE</b>GE <b>KE</b> ELELLLQA NKBVQYTT <b>TH</b> HSKCIRLG
121 GSSPE <b>AL</b>NSLKRQLQF M ENSRLFQNSLS <b>SL</b>DER <b>S</b> D IEGFLEEN QT KN SVF SVL DE
180 <b>ET</b> KALE BFWIE FGRKILKD LDTQ WSEK LK DLESFLRNLQD <b>SIKR</b> 225

(cAMP:1, CK2:2, PKC:1)
1 SNVVSRLSLFRLRS <b>IL</b> QNKCLF MNCRG ENL S VIK GMWD <b>SI</b> KSIL <b>T</b>V D E V <b>KS</b>DVF MG <b>T</b> IR
61 DE M <b>NTE</b> EL G K I L FM NC R G V Q TFLAVE MD KNYNTVG NFR T E A Q K MA QFLVGLKI 118

84kD (CK2:2, PKC:4)
1 LPRDSKCN M A S S <b>K</b> R S <b>IEPRK</b> IV <b>AKNN</b>N N M A S S <b>S</b>KSKSWMPADAR H T LNNV <b>T</b>M <b>R</b> QHS
61 TAPE <b>H</b>CRR PDPHCNLNRHNRHSTAPE <b>E</b>HCRRFIETSAAVQH <b>N</b> KWE <b>S</b>M PADA R HLMVQ
121 KDHLMVQKD P HCNLNRQTVGMQPMQQRWSMPADARHMTLNNV <b>T</b> MRQ GTVM Q MQPQQQRW A C
181 AVVF DCKFIETSS AAVQHNVKE201

43kD (cAMP:2, CK2:3)
1 CLSFARTYYGAILTLRTTTTLHGDLSQFHATVAERVRTFLHGDLSQFHATVAERVTILN
61 PQIMLSLYSLPEC <b>K</b> KTIILNPQIMLSYL <b>SP</b> ECK <b>K</b> KTY FLHGDLSQFHATVAERVA <b>F</b> EKI 119

39kD (CK2: 7, PKC:6)
1 LVQRGKKCYSWKDKLHGMKK <b>V</b> N E GG YI R LQF QYSA S LRA TFI Y FHKLGKL N C GKEF
61 YE KADA K E <b>ST</b> E TAHC SA VQQVAQKLVQRGTD <b>S</b> GP D D T YRS RLY AFIMHTLKHER AYA
121 FIMH TLKHER AEAPT SS SNS <b>T</b>STE ASGGSSEK GHI MLKH TGVKPHACQVCGK TE <b>ST</b> EET
181 A HKCGEC GMV FPRK EA PT SSS SNS <b>T</b>STE ASGGSSEK QGH M NKHLGVKPFQCQFCDFCKQH
241 MNKHLGVKPFQCQFCDKCSLQ E H M S I HTGES KYFC SIC GKS 281

33kD (CK2:1, PKC:2)
1 VIGKNGKVKHLECTKQLMLMSRN NSGVVRV RITKLMLMSRLQIDEQLRQE A<acronym>CA</acronym> N EAHKDQ
61 IG R S Y S<acronym>SG</acronym> GCTSTRVPE D L E RV IE GD NKE N KLEVE QLRM ERLMERLQIDEQLRQE ACANE
121 NAHKDFKKQLA AA FEEF VRELPRED G M VPFVFVG TKEG FLEVED F I QVPRNKVG
181 <b>VT</b> AI E LD E D TGFRI 195

30kD (CK2:1, PKC:3)
1 AQGVVLKVTENFK SQGDMLR <b>N</b> PINTKN<acronym>AF</acronym> HAALRNQAVKERA<acronym>T</acronym> LSSR FRRMARNT
61 LSS R FDIDE FDENKFQG DMLRAFHAALRNQ GDMLRAFHAALRNVD I D E F E N KFVDEHE
121 E A A AAGEPGDPCEVDGLLRQ 142
Fig. 6B

100kD (CK2:2)
1  GKGEGIRLSELRGGFNPVPNVEGRGEGLNEGFKFGECGCGESFNVMAMTEAAAKHAISM
61  TEAAAKHVQSERGLNEGFKFNNPVRG 87

98kD (CK2:2)
1  DLFTTRGEPNVAGLRVEETNLAGRGHSSHVEVLNAAGRRGGEQCCCHHHFNCGLGQIFVLSKDEV
61  TLNAGRGYEQCCHHFNCGLGQIFVLSKVD 88

85kD (CK2:8, PKC:11, Myristyl:9)
1  TRSEESSMPKSSASSSRKGLKICRTVREGPRSKQSLEDQKKEELDDFLFRKTAQPSPPR
61  GVTLGDTLTRLQLPEQSGPKKWAGASTAAQTKEQWAEREREGAGASTAAQKKEKAPFTS
121  ASVRGVSDESLVPPLAQKSVQAGNSDTEGGQPGRKSAPLPLTVVEELPAKGTALHGKVWP
181  Q5NPKGFGVQAGNSDTEGGQPGRKSRPGVSRDSNTSYTETKDAHALPEYSGKQADS
241  SSSRWSGASTAATQKPSISITTESLKECCEAEAPPATQPTSEQSILPESERT 299

69kD (CK2:4, PKC:3)
1  SHYMARIMASKGNSKKLAAEKSKKEQVGLMLTRTLQDELMTTRSKTELTEEERMVSVFY
61  AECRALSKRETITEEMLASQNSRLLLASQNILDEMVDELMTT 104

61kD (CK2:5, PKC:2, Myristyl:2)
1  PYAAARMVYGGPRYCLQLLAACDQKLQKAGHANFSARSAPPACFLKLPPQVAPRPKGL
61  DMPAATHWGLSGPHLSHGKASVRIRPPDSFDVPLLRWFLQAHQLRSLATVRYEAAPVDLLAPDPHSGREDCKPFAEGFCVQGVRGGDFIQVQGSAYEQ
121  HKIRRSLLGTEPSLHPAFRSCFVCALASLQEGPLSILLESYHEVLRCLVSLTPGSLEQ
181  PPTTHILPCTCRSWPSSHSSATSSVYDAHARIQ 241

53kD (CK2:3, PKC:1, Myristyl:4)
1  DRQTGKVVMRMNPDIMKAKNPDMKAPQRFLGEEKAFDADDEEFKKRLMDLLEEGLKRRAWNL
61  ICDSVCREAQTQSVAGGCYKLVISIDEAMMLQRAILDEHEKWKMLDLEEGLKRSGFDIL
121  GIKPVQRMRMLARLFPELQLQGNTAAAYLVAFTPQPPIDLQAFYKEFEDKGFQVQVD
181  DGRKSDGGYTLYSTDALAIQKVTGTVHVGVRGLGSKGETVRLMDLLEEGLKRYADF
241  SHNRNLNIFSDKMIYDIALDITLIERGESFYQDRM 276

45kD (PKC:5, Myristyl:5)
1  LIVVVRNLIVVVRNRVVTDKHRVVTDKGRPHPRVLHPDVRALHPDVARAQGSGRPRCAL
61  LEFLRLALLEFLRLTGPLPGLAVPEAVQVRLQDFGLKVRDFLKLRAISDQAYQL
121  SKTAQSGRPRCLGLSLQAYPFNFMRGLAWYRGLMPRTLAPGLPVASPGRPRLAPGL
181  PVAQSGPRRPLSHLFLVQGGRLPQVSVGEVRQDFFLGLKRAGGLGLGGQAGDLQQA
241  GTGQGTLRACPPAARAPVAPPAEPPHTSRL 275

34kD (CK2:3, PKC:2, Myristyl:2)
1  LEEERRREEEKKRLNVEKQVTSDKQVSGVRSLSKEEIERCFTPKGSLIKIQTENAFS
61  PSRSMVLEEEEQERRAEAGAPQQVEKRGGETENEFEKLRSEQYNALGDKTAKSGFSAPSA
121  SGTPNKEQTMFAPSSRSGRAINEWLTSFDGNSKMPEDGLSEDKKEFPKCGSFSAPSA
181  SGTPNKETAGLV 293

31kD (CK2:2, PKC:2)
1  TDPEAKVLLSCTHCKGLLTHCKGWGQQRLLIVHLKLVLVFVSVKISITNQISKDQVRMPNKSVD
61  RMPKSLVWFSVKSSTRVYSYVLVCKDVRMEMTEPLSPAASSQKELHVLKLYSVYLVCD 122
Fig. 6C

**179kD (CK2:4, PKC:2)**

1  
NNFEKKKNNFEGKKVAKPIWKKKICNDRIQHIAVFHRLECFWAAGDINCNDIRQKWQSVE  
DNASEPSGKEDKKEIYESTSSSWKRAASELAVSYAIDSLSKASSESLNNSCSPMEPLMLSSLE  
121  

**136kD (CK2:2, PKC:4)**

1  
QEEKKEFRPIKGWGDSKarenQDELEHRKQDEHELEKATGRNAVERR  
1  
MNTSAFPSRSLTSGEVARHYVPSGMSAKEEQEELKEVIERFSWKMRSSTKQ  
61  

**115kD (CK2:4, Myristyl:3)**

1  
ELTLKSTSEFAKAFVDPTDFKDEGVEEAARTIKEGVEEAARTVTDLGGFLSKTMLYVW  
ARCRDYKSEIEETVRMTYDEESDNVLRAlEESSDALLQKEARRKSTEMSEVLEIRVTAAFFAK  
99kD (PKC:2, Myristyl:5)

1  
GFPCLKKLIVVVRNRVVTDKHEGRPRVPTGKLPSFRAVPEAVQRALSIDAYATLKSQATQ  
GSGRPRLGWGSGTRALEFRRLLQAFYRFPNRRKTSFPFYTVQPVRPPFPHSFHFVSG  
121  
ERLAGSAGGAGAGDLDQAQG6TGQTALRAVPEAVQRIQLAQRFPNRRKMGSSGAGGAG  
181  
DLQGAGGTTGQRSTAFCQAPPAAPVPAPEPHTSSLR  
219  

**88kD (PKC:2, Myristyl:1)**

1  
CGPCKYESDIRVEASCIKQDAGDEREREEDGDGLKCAAACKHQKDYQDSARVHSKCGPCKYQ  
EQIDIHSSCNEILREGKSNCSELNLREDPGLYRDPVDQKDNQLGHTSSTDSKIM  
117  

**69kD (CK2:4, PKC:5)**

1  
THSDDTRFAPYDKKIKLEFKKEEVLLKWYLVSHFESQELSKASKEDFNEQDKKALKAMSL  
LSSRNSKTVGLYLRICLLCHPGPPGTGSFSLDSQVNHRIEVEKVRQPAKAAEIATYAM  
121  
VKHLLEENVEQNMIGRLGCAPTLSQISRPRPVRFAECSCQCIQTQTDLPSVKFNFCLLL  
219  
EENVEQNMIGRLAQEGQPYQDSMMARFRVQRRMSARFVQLSILQVQARFQHTQPS  
241  
SSSKQSCCLTFSENRPTASAPVNLPSQSFIDTFIKE  
280  

**58kD (CK2:3, PKC:2, Myristyl:1)**

1  
SNVEKAADVNTIKSVMCNTKSMNHQKIQGILGRWSKQEQQOLQAKSLRQHLEKFLGR  
SNVEHAKDKDSLDDSSPAKEQKQQLIQOAKIKILQLAFPDMNHNSIKINSSSHKRPMA  
121  
FMVWAKDQLHSHSAGALENSPNTPRFKDLTATHEQKRNAMAMLFEKQ  
168  

**49kD (PKC:7, Myristyl:1)**

1  
LFPSKCATLPRQHDEVPPKVGYDERSKSSKSTHPQRAGAPGTLTSKRDLDQGRGPGAPGP  
DREDGQLSPPPGSHPSPGDSQSLPLTVKQPQAPQPBPSPPSPSSAHSKPSRNLDPLQELAQR  
121  
ILEETKSLASSDTGSEDQSSTSTTDSKQSLSPUTVKQPPARSSSPLLKV  
171  

**42kD (CK2:1, PKC:2, Myristyl:2)**

1  
GPRGDPAAARAAAPLAVSRIRAAPLVASRVIDGDPEPAHHGAGPAAARRWIIWLRGLGVLAA  
AVRAMKTHGETNLWLVGPCHHRIDDGPLPAHHGAGPAARRWRAVGAPGPAEAEEVDVAAA  
121  
LPARKVGAGPQAEAVGEVDVAAALPAARGAARG  
155  

20
Fig. 7

KD Marker ERβ TAD LBD
200 150 100 85 70 60 50 40 30 25 KD Marker ERβ TAD LBD
200 150 100 85 70 60 50 40 30 25 KD Marker ERβ TAD LBD
200 150 100 85 70 60 50 40 30 25

A (CK2) B (PKC) C (N-Myrstyl)
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