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FKBP Family Proteins: Immunophilins with Versatile Biological Functions

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Key Words

Immunophilin • FK506-binding protein • Peptidylprolyl *cis/trans* isomerase • Immunophilin ligand • Neuroprotection • FK506 • Rapamycin

Abstract

Immunophilins consist of a family of highly conserved proteins binding with immunosuppressive drugs such as FK506, rapamycin and cyclosporin A. FK506-binding protein (FKBP) is one of two major immunophilins and most of FKBP family members bind FK506 and show peptidylprolyl *cis/trans* isomerase (PPIase) activity. Small size FKBP family members contain only FK506-binding domain, while FKBPs with large molecular weights possess extra domains such as tetratricopeptide repeat domains, calmodulin binding and trans-membrane motifs. FKBPs are involved in several biochemical processes including protein folding, receptor signaling, protein trafficking and transcription. FKBP family proteins play important functional roles in the T-cell activation, when complexed with their ligands. The roles of immunophilins in protein transportation and apoptosis through their molecular interactions with receptors or proteins have emerged recently. Moreover, therapeutic implications of immunophilin ligands in treating neurodegenerative disorders have been accumulating. FK506 and its derivatives with no immunosuppressive activities bind to the conserved active sites of the canonical FKBP members such as FKBP12, which shows PPIase activity. These immunophilin ligands show variable efficacy in animal models for Parkinson's disease, dementia, and spinal cord injury, where the canonical immunophilins function as chaperones and are associate with the protein folding and modulation of oxidative stress. On the other hand, in the noncanonical FKBP members such as FKBP38, FK506-binding site is not conserved and shows neither PPIase activity nor affinity to FK506. Interestingly, the small molecule-mediated inhibition of the noncanonical member of FKBP family appears to cause neuronal protection and induce proliferation of neuronal stem cells in a rat focal cerebral ischemia model. Currently, the mechanisms of actions remain unclear. This review focuses on molecular characteristics of the canonical and noncanonical FKBP family members and the biological functions of their ligands in performing neuroprotective and neurotrophic activities.

Introduction

FK506 (tacrolimus), rapamycin (sirolimus), and cyclosporine A (CsA) are well-known immunosuppressive drugs that bind to immunophilins, which exhibit peptidylprolyl *cis/trans* isomerase (PPIase) activity [1–3] . FK506-binding proteins (FKBPs) with various molecular weights are the principal intracellular targets for FK506 and rapamycin, while CsA binds to cyclophilins (CpN) [3] . The formation of FKBP/FK506 and CpN/CsA complexes inhibits not only the PPIase activity of FKBP and CpN, respectively, but also the phosphatase activity of the secondary target calcineurin (CaN), thereby preventing the dephosphorylation of NF-AT that is required for IL-2 gene expression and T-cell activation [4, 5] . On the other hand, rapamycin binds to FKBP12, but the FKBP/rapamycin complex interacts with mammalian target of rapamycin (mTOR) instead of CaN and exerts immunosuppressive activity [6] (fig. 1).

Despite the role of FKBPs and CpNs in modulating T cells, interestingly the immunophilins are more abundant in nervous tissues than in immune tissues, suggesting their biological significance in neurons [7]. Indeed these immunophilin ligands exert neuroprotective and neurotrophic effects. In this review, we provide an overview on the immunophilins in mediating diverse biological functions and examine their molecular characteristics by using examples of the canonical members such as FKBP12 and noncanonical members such as FKBP38, 51 and 52.

FKBP as PPIase

In folded proteins there are two different conformations of peptide bonds, *cis* or *trans* . Most peptide bonds are found in *trans* conformation in folded proteins, whereas 6% of all Xaa-Pro peptide bonds show *cis* conformation [8]. In vitro studies have shown that energetically-hindered isomerization of Xaa-Pro bonds may limit refolding process of proteins [3]. The *cis/trans* peptidylprolyl isomerization is catalyzed by a super family of PPIase (EC 5.2.1.8) [9, 10]. PPIase of FKBPs are involved in the slow protein folding process and conserved in all organisms from *Archaea* bacteria to primate [3, 11]. *Cis/ trans* interconversion of Xaa-Pro peptide bond occurs through binding of a peptide substrate in the hydrophobic binding pocket of the PPIase. The isomerization is facilitated by out-of-plane conformational change of amide bond, which is stabilized through a hydrogen bonding to the amino acid located in the hydrophobic binding cleft, resulting in stabilizing the transition state during the isomerization process [3]. PPIase interacts with a diverse range of intra- and extracellular targets, but the molecular interactions between PPIase and target proteins with Xaa-Pro sequences are weak. Thus, the amount of PPIase required for performing catalysis is usually high.

FKBPs and Chaperone Activity

Chaperones are proteins that can recognize non-native proteins, prevent unwanted inter- and intramolecular interactions and influence the partitioning between the productive and unproductive folding steps. The chaperones are saved and excluded from the final structures of the folded proteins. There are many examples of FKBPs functioning as chaperones, such as

the mammalian FKBP52, the wheat FKBP73 and *Archaea* bacteria FKBP [3, 12]. Recently, human FKBP38 and *Plasmodium falciparum* FKBP35 (PfFKBP35) have been shown to exhibit chaperone activity [13, 14]. The chaperone activity of FKBPs is not inhibited by FK506 or rapamycin, suggesting that this activity is independent of the PPIase activity.

FKBP12 and Its Function

FKBP12, a prototype FKBP which is an extensively characterized member in FKBP family, contains only a single FK506-binding domain (FKBD) comprised of 108 amino acids. It is abundantly and ubiquitously expressed with PPIase activity. Human FKBP12 interacts with FK506 with a K_D of 0.4 nM or rapamycin with a K_D of 0.2 nM [15]. The complex formations of FKBP with the ligands enhance the stability of FKBP and the resulting complexes remain more resistant to proteolytic cleavage and create an appropriate binding surface for binding to CaN and mTOR, respectively [3, 16] . Interestingly, in the absence of FK506, FKBP12 binds to cellular targets such as modulate ryanodine receptors (RyRs), which is one of the major Ca^{2+}-releasing channels in the sarcoplasmic reticulum [12, 17, 18] . The molecular interaction between FKBP12 and RyRs stabilizes the RyR channel and modulates channel gating by increasing the number of RyRs at the full conductance level and thus mean open time [17, 19–21] and the removal of FKBP12 from RyR channels inhibits coupled gating [22] , implying an important role of FKBP12 in modulating RyR complexes. FK506 or rapamycin causes a Ca^{2+} leakage in isolated endothelial cells and induces an intracellular Ca^{2+} leakage that may contribute to the pathogenesis of endothelial dysfunction and hypertension [23]. FKBP12 has also been demonstrated to tightly interact with the inositol 1,4,5-trisphosphate receptor (IP_3R) which is activated through phosphorylation by protein A kinase and inactivated through dephosphorylation by CaN [12] . The binding of FKBP12 to IP_3R enables it to interact with CaN and possibly modulate the receptor's phosphorylation status [24, 25].

FKBP12 also acts as a natural ligand for transforming growth factor-β (TGF-β) that regulates a wide range of biological processes. FKBP12 binds to glycine- and serine-rich motif (GS motif) of TGF-β receptor I (TGF-βRI), capping its phosporylation and further stabilizes the inactive conformation of TGF-βRI [26] . The PPIase core domain of FKBP12 is important for the interaction and FK506 inhibits the interaction between the two proteins, suggesting that FK506 and TGF-βRI share a common binding site on FKBP12. Activin, a member of TGF superfamily, induces the dissociation of FKBP12 from the Activin type I receptor (ALK4) and thus exerts its signal. FKBP12 interacts with another inhibitory molecule of Activin signal, Smad7, in an Activin-dependent manner [27] and associates again with ALK4 to suppress the Activin signal. FKBP12 has also an inhibitory effect on epidermal growth factor receptor autophosphorylation [28]. The PPIase activity and the hydrophobic drug-binding pocket of FKBP12 seem to be important for the interaction between FKBP12 and the receptors. Together, these data suggest that FKBP12 is involved in protein-protein interactions and regulates the activities of its cellular partners.

FKBPs with Multi-Domains

Among FKBP family members, FKBP38, 51, and 52 all consist of a single FKBD followed by additional functional units [3]. FKBP38 is a multifunctional protein and contains FKBD,

tripartite TPR domain, putative calmodulin (CBD) and transmembrane (TM) motifs [29, 30] (fig. 1). FKBP38 helps anti-apoptotic proteins Bcl-2 and Bcl-X_L to localize at the mitochondrial membrane and protects cells from apoptosis [14, 30]. How does FKBP38 interact with Bcl-2? Three-dimensional structural studies reveal that Bcl-2 contains a long flexible loop between Bcl-2 homology 3 and 4 (BH3 and BH4) regions [31]. FKBP38 binds to the flexible loop of Bcl-2 and protects Bcl-2 from degradation [32]. In HEK293 and MEF cells, however, FKBP38 can play an additional chaperone role for Bcl-2 to change its destination to ER membrane through its interaction with Presenilins 1 and 2 (PS1/2) and Bcl-2 [33]. FKBP38 interacts with HSP90 through TPR domain. The interaction between FKBP38 and HSP90 not only inhibits the PPIase activity of FKBP38 but also the interaction between FKBP38 and Bcl-2 [34]. When FKBP38 is knocked down by small interfering RNA, the level of Bcl2 protein is also significantly reduced, while no apparent change in the level of Bcl-2 mRNA is observed. This result suggests that the molecular interaction between FKBP38 and Bcl-2 is important for the function and stability of Bcl-2 and protects the anti-apoptotic protein from potential protein degradation pathways.

The phosphatase activity of CaN is inhibited by forming a ternary complex with FK506 and FKBP12 [35]. Interestingly, after the initial report showing FKBP38 as an endogenous inhibitor of CaN was made, contradictory observations about the inhibitory effect of human FKBP38 on CaN have been reported [30, 36]. A nuclear magnetic resonance spectroscopy study demonstrated that upon the addition of FK506, no apparent spectral changes were detected in $2D^{1}H$-^{15} N heteronuclear single quantum correlation (HSQC) spectrum of FKBD of FKBP38, while chemical shift perturbations were observed in the HSQC spectrum of FKBP12 upon the addition of FK506 [14]. This study clearly suggests that FKBP38 has no affinity to FK506 and the mode of action differs from those of canonical FKBP family members. At least in vitro no intrinsic CaN inhibitory activity of FKBP38 was detected. However, since the observations were made in two different experimental conditions, whether FKBP38 functions as an endogenous inhibitor of CaN in the absence of FK506 remains to be further investigated.

Recently, Rheb, a Ras-like small guanosine triphosphatase (GTPase), in response to growth factor stimulation and nutrient availability, interacts directly with FKBP38 in the absence of rapamycin and prevents its association with mTOR in a guanosine 5-triphosphate (GTP)-dependent manner, suggesting that FKBP38 is an endogenous inhibitor of mTOR [37]. A previous study also suggests FKBP38 as an intrinsic inhibitor of CaN [30]. Together these data indicate that the molecular mechanism of the noncanonical FKBP family member FKBP38 is unique and pleiotrophic.

Unlike FKBP38, larger-sized FKBP family members FKBP51 and 52 possess PPIase activity and bind FK506 [38–41]. These proteins contain a tandem FKBD separated by a short linker sequence. The N-terminal FKBDs of the larger FKBP family members are responsible for the PPIase- and ligand-binding activities. The C-terminal FKBDs are inactive in those activities. The latter domains contain an ATP/GTP-binding sequence [40, 42]. Thr143 residue located between the two FKBDs is phosphorylated by casein kinase-II [43]. Similarly, TPR domains are also important for their interactions with heat shock protein 90 (HSP90) [40]. The resulting FKBP51/ HSP90 or FKBP52/HSP90 complexes are associated with progesterone

receptor (PR) or glucocorticoid receptor (GR) and the ternary complexes migrate from the cytoplasm to the nucleus [44].

Structural Characteristics of FKBPs

The prototype FKBP family member FKBP12 only contains a single FKBD (fig. 2), which is responsible for both PPIase- and FK506-binding activities. The structure of FKBP12 is characterized by an amphipathic five-stranded β-sheet. The β-sheet has a right-handed twist and wraps around the helix, and forms a hydrophobic pocket in which FK506 or rapamycin binds between the α-helix and β-sheet (fig. 3a, c).

FKBP38, which is a noncanonical FKBP family member, unlike most canonical FKBPs, lacks the conserved amino acid residues required for binding FK506 and for PPIase activity, suggesting its FK506-independent function [14, 29, 30]. FKBP38 also lacks the well-conserved Trp59 in FKBP12 which is important for the interaction with FK506 and has instead Leu residue at the corresponding position [14, 29]. NMR solution structures reveal that the FKBD of FKBP38 shows the overall structural similarity to that of FKBP12 [45, 46]. However, the hydrophobic packing and interactions could be unfavorably influenced by the multiple substitutions of the aromatic residues in FKBP12 to Leu in FKBP38, since the Leu provides a smaller van der Waal surface than that generated by aromatic residues (fig. 3b, d). This could be a molecular basis by which FKBP38 has no affinity to FK506.

It is interesting to note that FKBP38 appears to contain the secondary structure elements of an 'inactive' FKBP-type isomerase. In other words, it lacks significant loop insertion between β3 and β4. The larger molecular weight FKBP family proteins FKBP51 and 52 show unique structures featuring in tandem FKBDs and multiple TPR domains [41, 44]. Their N-terminal FKBD domains (referred to as FKBD1 in figure 2) possess PPIase activity and can bind FK506 and rapamycin. On the other hand, the C-terminal FKBP domains (referred to as FKBD2 in figure 2) neither possess measurable rotamase activity nor binds FK506 and rapamycin. The FKBD2 is mainly responsible for the molecular interactions between the immunophilins and proteins such as HSP90 and steroid receptors [40, 41, 47, 48]. Structures of FKBD1 and FKBD2 of FKBP51 [41] and FKBP52 [44] reveal that the amino acid substitutions at the active site and loop insertions flanking the binding pocket directly cause the loss of activity in the FKBD2. The structure of FKBP38 more closely resembles the C-terminal rather than the N-terminal FKBD domains of these two family members. However, the binding surface of FK506 is partially preserved in FKBP38 and not optimal for the full rotamase activity compared to canonical FKBP family members. FKBP38 retains a noncanonical rotamase activity, which is activated in response to Ca^{2+}/calmodulin mobilization, in an unknown mechanism [49].

Role of FKBPs in Neuronal Cells

FKBPs are abundantly present in neuronal tissues and their expressions are elevated after nerve injury [7, 50, 51], leading to examine the roles of FKBP ligands in neuronal cells. As alternative therapeutics to protein neurotrophins for the treatment of neurodegenerative diseases, immunosuppressive drugs have been studied and shown to have variable effects in

reversing neurodegenerative process and preventing apoptotic cell death in neuronal cells [52–54]. However, in contrast to neurotrophins, most immunophilin ligands are highly stable and can readily cross the blood-brain barrier, prompted the development of nonimmunosuppressive immunophilin ligands with potent and selective therapeutic activities. GPI-1046, one of nonimmunosuppressive immunophilin ligands, binds to FKBP12, and like FK506, it showed neuroprotective and neuroregenerative effects on primary cultures of midbrain dopaminergic neurons against both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) toxicities [55], while exerting similar effects in rodent models [56, 57]. GPI-1485, the second-generation compound of GPI-1046, demonstrated substantial efficacy in preclinical and initial phases of clinical evaluation for Parkinson's disease (PD) model [58]. Another V-13,661, which does not bind FKBP12, can prevent a progressive dopaminergic axonal degeneration and neuronal death in a mouse model [59]. A recent study also demonstrates that the inhibition of the PPIase activity of FKBP38 by a small molecule inhibitor N-(N',N'-dimethylcarboxamidomethyl)cycloheximide shows neuroprotection as well as neural stem proliferation in a rat model for an acute focal cerebral ischemia [60] , adding an additional diversity of the immunophilin-related neuroprotective mechanism.

How do these immunophilin ligands exert neurotrophic and neuroprotective functions? The canonical FKBP family members bind FK506 and the resulting FKBP/FK506 complexes inhibit CaN activity, leading to immunosuppression. However, the ligand binding does not appear to be required for the effects of the immunophilins on neurons, since both immunosuppressive and nonimmunosuppressive immunophilin ligands and also compounds that have no affinity to FKBPs [59] or even FKBP12 knock-out mice still show efficacies in neurons [55]. These data suggest that neurotrophic and neuroprotective functions of immunophilin ligands can occur without forming complexes between immunophilin ligands and immunophilins. FKBPs also bind to other chaperone proteins and receptors such as HSP90 [41], steroid receptor [17], and regulatory molecules [26, 30], suggesting important biological function of FKBPs in protein-protein interaction in various signaling pathways. FKBPs possess PPIase activity, function as chaperone proteins, and may aid protein folding of target molecules [52]. Or the neuroprotective properties of FKBPs may be associated with modulation of oxidative stress in neural tissues [61]. Thus, the immunophilin ligands-mediated neuroprotective mechanism appears to be complex and might depend on combination of various external stimuli and cellular signaling pathways.

Conclusion

FKBPs in mammals and other organisms play important roles in various biochemical processes including protein folding, protein trafficking, and protein assembly. Accumulating evidence no doubt suggests that natural immunosuppressive drugs FK506, CsA, rapamycin and their analogs can promote neurite outgrowth and neuronal survival in in vitro and in vivo animal models and provide new therapeutic windows for neurodegenerative disorders such as PD. Several studies demonstrated that the complex formation between immunophilin ligands and immunophilin may not be necessary for neurotrophic and neuroprotective functions of immunosuppressive drugs [60], suggesting the presence of potential and novel mechanisms of actions of these drugs. Recent studies have shown that FKBP12, 38 and 52 are abundantly

expressed in the brain and participate in multiple cellular processes [60, 62–64]. With three-dimensional structural data available for FKBP family members, recent findings on FKBP family would certainly lead to the structure-based rational design of selective ligands that target specific FKBP family members in response to different external stimuli. The promising leads in this therapeutic approach would minimize the potential side effects associated with the lead ligands.

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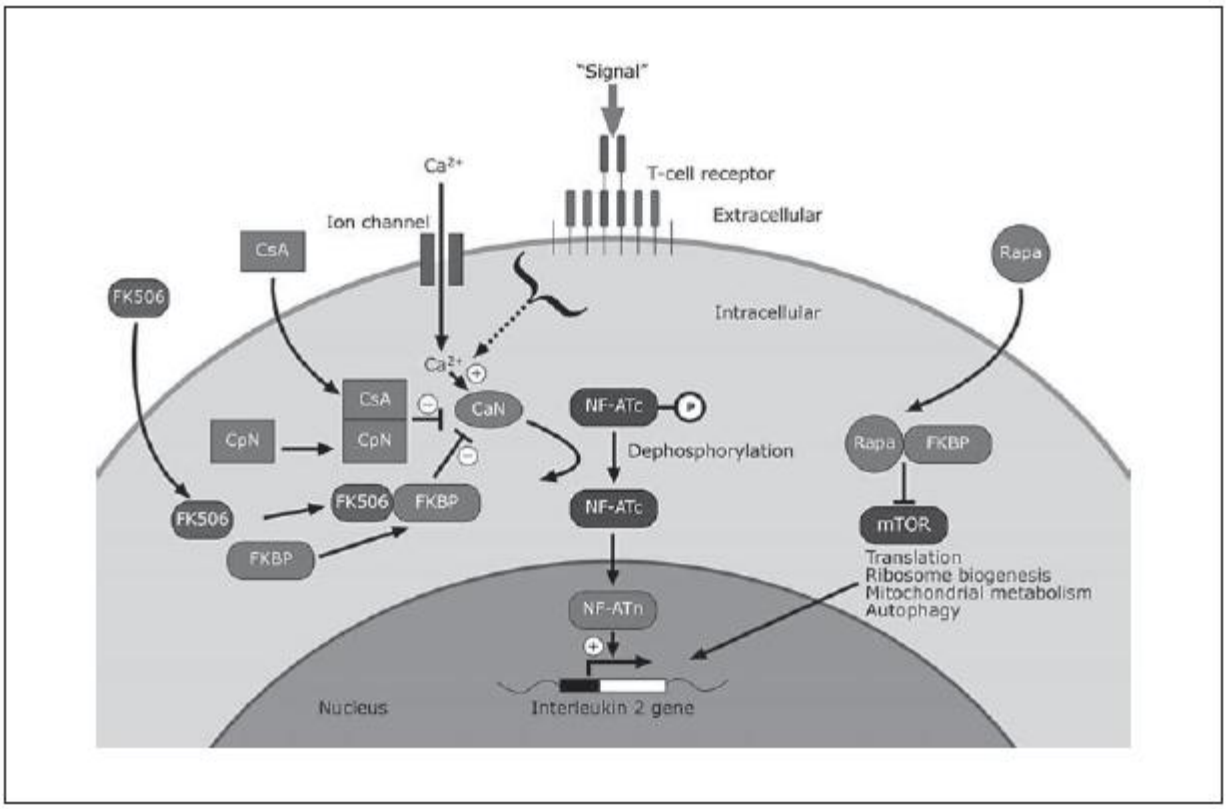
List of Figures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NF-ATc
Dephosphorylation
Rapa
FKBP
FK506
FK506
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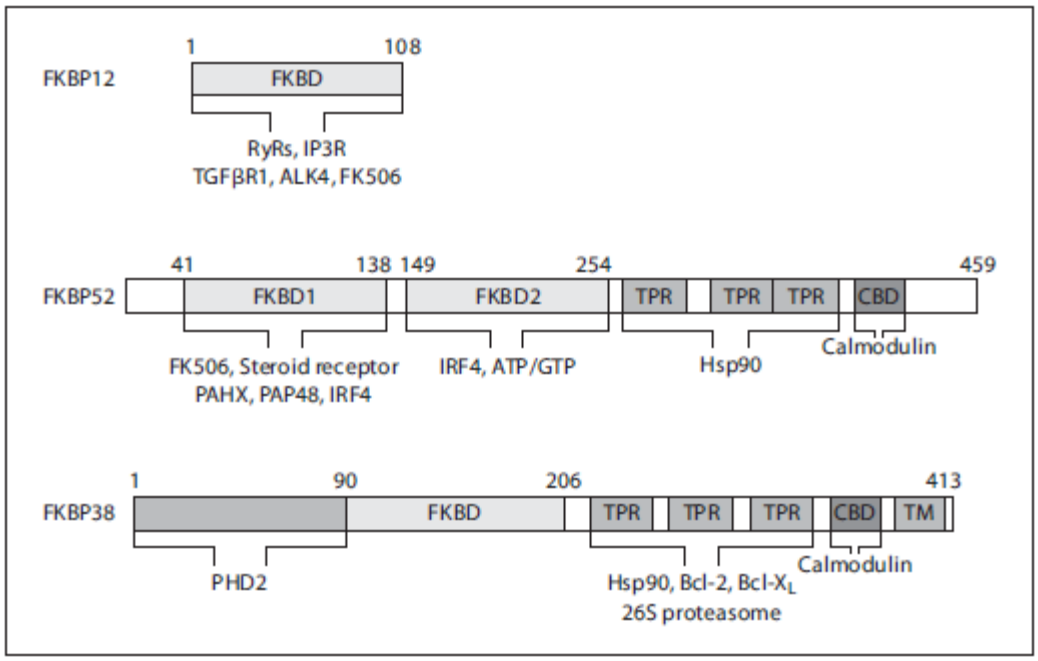
mTOR
Translation
Ribosome biogenesis
Mitochondrial metabolism
Autophagy
NF-ATn
Nucleus
Interleukin 2 gene

Figure 1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TPR
TPR
CBD
FK506, Steroid receptor
PAHX, PAP48, IRF4
IRF4, ATP/GTP
Hsp90
Calmodulin
FKBP38
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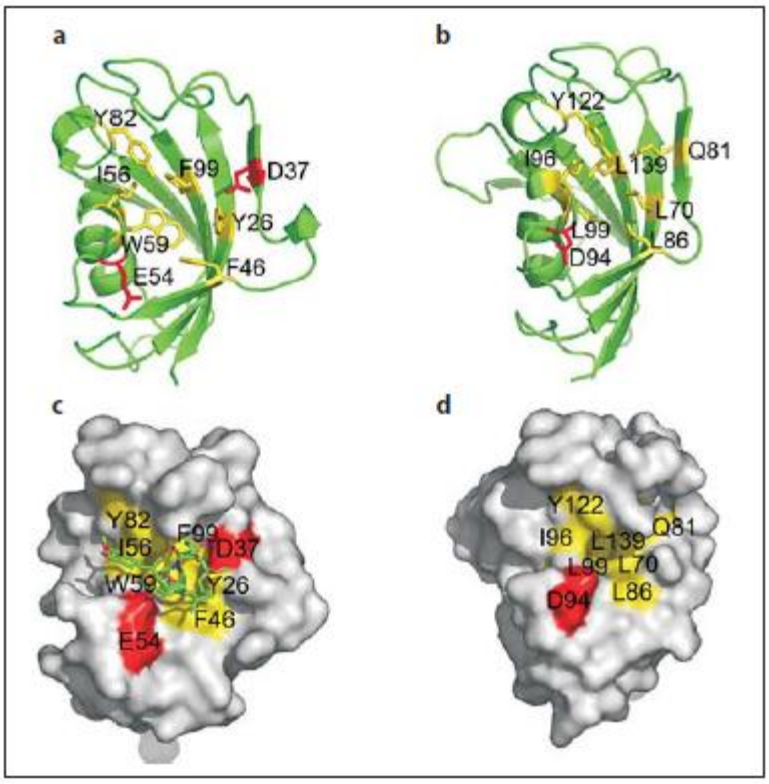
PHD2
Hsp90, Bcl-2, Bcl-X$_L$
26S proteasome
Calmodulin

Figure 2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d
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L139
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L99
L70
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D94

Figure 3